Abstract

**Purpose:** Clinical and epidemiologic data suggest that obesity is associated with more aggressive forms of prostate cancer, poor prognosis, and increased mortality. C-terminal–binding protein 1 (CtBP1) is a transcription repressor of tumor suppressor genes and is activated by NADH binding. High calorie intake decreases intracellular NAD⁺/NADH ratio. The aim of this work was to assess the effect of high-fat diet (HFD) and CtBP1 expression modulation over prostate xenograft growth.

**Experimental Design:** We developed a metabolic syndrome-like disease in vivo model by feeding male nude mice with HFD during 16 weeks. Control diet (CD)–fed animals were maintained at the same conditions. Mice were inoculated with PC3 cells stable transfected with shCtBP1 or control plasmids. Genome-wide expression profiles and Gene Set Enrichment Analysis (GSEA) were performed from PC3 shCtBP1 versus PC3 pCIPZ HFD-fed mice tumors.

**Results:** No significant differences were observed in tumor growth on CD-fed mice; however, we found that only 60% of HFD-fed mice inoculated with CtBP1-depleted cells developed a tumor. Moreover these tumors were significantly smaller than those generated by PC3 pCIPZ control xenografts. We found 823 genes differentially expressed in shCtBP1 tumors from HFD-fed mice. GSEA from expression dataset showed that most of these genes correspond to cell adhesion, metabolic process, and cell cycle.

**Conclusions:** Metabolic syndrome–like diseases and CtBP1 expression cooperate to induce prostate tumor growth. Hence, targeting of CtBP1 expression might be considered for prostate cancer management and therapy in the subset of patients with metabolic syndromes. *Clin Cancer Res; 20(15); 4086–95. ©2014 AACR.*

Introduction

Prostate cancer continues to be a major health care problem worldwide (1). Epidemiologic studies indicate that elevated body mass index (BMI) correlates with elevated risk of prostate cancer-specific mortality and biochemical recurrence (2). Because of the increase of overweight and obesity incidences throughout the world, the number of men at risk for developing prostate cancer is also on the rise. There are several molecular mechanisms that physiologically link obesity to cancer risk; however, it remains a puzzle how exactly obesity activates the transformation pathways that result in cancer.

Calorie excess intake impacts life span, the incidence of diseases, and metabolic disorders through diverse mechanisms (3). For instance, nutrient excess influences NAD⁺/NADH ratio with the associated increase of ROS as a consequence of incomplete mitochondrial electron transfer during respiration. High ROS influences cell proliferation, differentiation, and death, as these processes are intrinsically dependent upon the cellular redox status. In addition, ROS also contribute to increase the risk of malignant transformation by causing DNA damage (4). Calorie excess affects NAD⁺ availability, which in turn, modulates the activity of certain classes of mammalian proteins that utilize NAD⁺ or NADH as cofactors, ligands, or substrates. Some of these molecules include the Sir2 family of Class III histone deacetylases, the PARP family of poly...
and progression. In addition, the regulation of this function suggests that CtBP1 might be crucial in tumor negative regulator of several important tumor suppressors. CtBP1 interacts with a broad range of transcription factors and represses transcription of several tumor suppressor genes such as E-cadherin, BRCA1, CDKN2A, Sirtuin 1 (SIRT1), and PTEN (6, 8–11). Therefore, since the discovery of CtBP1, several efforts were made to assess the involvement of CtBP1 in cancer development (12, 13).

Previously, Di and colleagues showed that NAD+/NADH levels selectively regulate BRCA1 tumor suppressor gene (8). The release of CtBP1 from the BRCA1 promoter through estrogen induction or enhanced NAD+/NADH ratio leads to HDAC1 dismissal, elevated histone acetylation, and increased BRCA1 transcription, diminishing cancer risk (8).

CtBP1-mediated transcriptional repression of E-cadherin seems to be regulated by the hypoxic environment in solid tumors with poor vascularization and high metabolic activity. A hypoxic condition that increases free NADH levels has been shown to enhance recruitment of CtBP1 to the E-cadherin promoter inducing tumor cell motility. Hence, CtBP1 was suggested to participate in the epithelial-to-mesenchymal transition (6).

In summary, literature fosters a strong role for CtBP1 as a negative regulator of several important tumor suppressors. This function suggests that CtBP1 might be crucial in tumor initiation and progression. In addition, the regulation of CtBP1 by NAD+/NADH places this factor in the category of molecules that link transcription to cellular metabolism. Nevertheless, the importance of CtBP1 as a sensor of nuclear redox state in vivo has yet to be determined. Our hypothesis was that enhanced CtBP1 activity in prostate tissues with low NAD+/NADH ratios, as a consequence of high calorie intake, contributes to increase prostate tumor development. Here, we explored CtBP1 role in prostate malignant transformation. Using an in vivo model of reduced NAD+/NADH ratio, we investigated the effect of the high-fat diet (HFD) on prostate tumor growth through CtBP1 modulation. We focused our studies in the molecular pathways regulated by CtBP1 and the interplay with sexual hormones in prostate cancer.

Materials and Methods
Cell culture, transfections, and treatments
PC3 (ATCC: CRL-1435), 22Rv1 (ATCC: CRL-2505), LNCaP (ATCC: CRL-1740), and C4-2 (14) prostate cancer cells were grown in RPMI-1640 (GIBCO) with 10% FBS in a 5% CO2 humidified atmosphere at 37°C. PC3 stable cell line (PC3.CtBP1) was generated by transfecting with pcDNA3.CtBP1 plasmid (15 μg, Origene) and Lipofectamine 2000 (Invitrogen) as previously described (15). PC3 CtBP1-depleted cells (PC3.shCtBP1) lentiviral infection was performed as described in Supplementary Methods.

PC3 cells were exposed to testosterone undecanoate (10 μmol/L) or DMSO as vehicle in phenol red-free RPMI-1640 medium (GIBCO) supplemented with 10% charcoalated FBS. Letrozole (Femara-Novartis) was prepared in ethanol and PC3 cells were exposed for 1 hour (5 μmol/L) previously to testosterone stimulation and incubated for 24 hours. PC3 cells were transfected with Lipofectamine 2000 and 1 μg of AR5 vector (gently gifted by Dr. G. Jenster, Erasmus University Rotterdam, the Netherlands).

Chromatin immunoprecipitation (RT-qPCR, Western blot analysis, and IHC)
Chromatin immunoprecipitation (ChIP), RT-qPCR, Western blot analysis, and IHC were performed as described in Supplementary Methods (15–17).

Focus formation and clonogenic assays
NIH3T3 cells were transfected with 5 μg pcDNA3.CtBP1 or control (pcDNA3.β-Gal) plasmids by calcium-phosphate method as previously described (18). For clonogenic assays, 103 cells were seeded in a 100-mm plate and incubated for 2 weeks. In both methods, cells were methanol fixed and stained with crystal violet. Foci number and area were quantified using GelPro Analyzer v4.0 software. Photographs were acquired with Phosphorimager (Fuji Photo Film Co. Ltd.).

PC3 high-fat xenograft model
All animal experiments followed the institutional guidelines for animal welfare. Four-week-old athymic male Swiss nu/nu mice were fed for 16 weeks with control diet (CD) (n = 18) or HFD (n = 18). Chow food was supplemented with bovine fat in a 2:1 proportion (w/w) to generate HFD. CD and HFD had 3 or 5 kcal per gram of food, respectively. After 12 weeks of diet, each CD- and HFD-fed mice group were randomly divided and subcutaneously injected with PC3, pGIPZ or PC3.shCtBP1 cells (4.8 × 106). Body weight was determined three times a week. Tumor size was measured...
for 4 to 6 weeks and tumor volume was calculated as described (19). At necropsy, blood was drawn from all mice by direct heart puncture; serum was separated and tumors were excised. Tissues were formalin fixed and paraffin embedded. Histopathology and IHC studies were performed in 5 μm tissue sections using hematoxylin and eosin (H&E) or specific antibodies: anti-CtBP1 (BD Biosciences); anti-BRCA1 (ARP33338, P050, Aviva System Biology), anti-E-cadherin (Clone HECD-1, Zymed Laboratories Inc); and anti-cyclin D1 (H295, Santa Cruz Biotecnoologies).

Serum cholesterol, triglycerides, glycemia, NAD+/NADH levels, testosterone, and estradiol determinations were performed as described in Supplementary Methods.

Microarrays

Microarrays were performed as described in Supplementary Methods (20).

Statistical analysis

All results are given as mean ± SD of three independent experiments. Student t tests were used to ascertain statistical significance with a threshold of P < 0.05. For in vivo experiments, two-way ANOVA followed by Dunnert test were performed. Shapiro–Wilk and Levene tests were used to test normality and homogeneity of variances. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Results

CtBP1 is overexpressed in human prostate tumors

We analyzed CtBP1 expression on a small group of 20 radical prostatectomies specimens and found that normal, benign, and carcinomatous epitheliums showed different positive CtBP1 immunoreactivity patterns (Fig. 1A). Normal glands columnar epithelia and some basal cells showed a mild positive nuclear stain (Fig. 1A), whereas in benign prostatic hyperplasia, the columnar epithelium showed a moderate positive nuclear immunostain (Fig. 1A). Remarkably, all the cells in the epithelia of the carcinomatous glands showed intense nuclear and moderate cytoplasmatic immunostaining (Fig. 1A).

In accordance with these findings showing that CtBP1 expression is increased in human prostate cancer samples, recently, Wang and colleagues (12) observed CtBP1 overexpression and mislocalization in human metastatic prostate cancer.

CtBP1 increases transformation and proliferation in vitro

To investigate whether CtBP1 induces transformation in vitro, we performed the focus formation assay transfecting NIH 3T3 cells with pcDNA3.CtBP1 or control vectors. We found that CtBP1-transfected cells showed larger and higher...
number of foci compared with control (Supplementary Fig. S1A).

Next, we determined CtBP1 expression in prostate cancer cell lines (Fig. 1B). We found that androgen-sensitive LNCaP cells showed higher CtBP1 expression compared with the other prostate cancer lines. Notably, androgen-insensitive PC3 cells showed five times lower CtBP1 expression than LNCaP cells (Fig. 1B). We generated stable transfected PC3 cells with enhanced (PC3.CtBP1) or depleted (PC3.shCtBP1) CtBP1 expression, as confirmed by Western blot analysis and RT-qPCR compared with PC3.pGIPZ control cells (Fig. 1C and Supplementary Fig. S1B).

To determine CtBP1 role on cell proliferation, we assessed stable transfected PC3 cells clonogenic capacity. PC3.shCtBP1 cells showed significantly lower colony number compared with control (Fig. 1D). On the other hand, CtBP1 overexpression increased not only the colony number but also the colony area (Fig. 1D). Altogether, these results indicate that CtBP1 induces transformation of NIH 3T3 cells and proliferation of prostate tumor PC3 cells in vitro.

HFD induces metabolic syndrome–like disease in nude mice

On the basis of high NADH levels induce CtBP1 dimerization and activation (7), we investigated whether HFD influences NAD+/NADH ratio and, in turn, tumor growth using a murine xenograft experimental model. Males nu/nu mice fed with HFD or CD during 12 weeks were subcutaneously inoculated with PC3.pGIPZ or PC3.shCtBP1 stable cell lines. After 4 to 6 weeks from cell inoculation, mice were sacrificed. Despite HFD-fed mice showing a trend toward gaining body weight, no significant differences were detected among mice fed with HFD or CD along the experiment (Fig. 2A). Nevertheless, animals fed with HFD presented hypercholesterolemia at the end of the experiment (Fig. 2B), with no significant changes in serum triglycerides and glycemia compared with control mice (Supplementary Fig. S2A and S2B).

As a consequence of a prolonged HFD intake, animals developed a metabolic syndrome–like disease, evidenced by hormonal imbalance and kidney and liver histologic architecture disorganization. We found significant decreased testosterone serum levels in HFD mice (Fig. 2C) with no changes in estradiol serum levels (Supplementary Fig. S2C).

Furthermore, kidneys from HFD-fed mice showed glomeruli significantly enlarged by mesangial hypercellularity and edema at the epithelium of the collecting duct (Supplementary Fig. S2D), whereas liver displayed altered architecture of hepatic lobules and a pronounced vacuolization of hepatic cells as a consequence of steatosis (Supplementary Fig. S2E).

CtBP1 depletion decreases tumor growth in HFD-fed mice

We assessed tumor growth in a xenograft model injected subcutaneously with PC3.shCtBP1 or PC3.pGIPZ cells. As shown in Fig. 3A, nonsignificant differences were observed in the tumor growth curves from PC3.shCtBP1 and PC3.pGIPZ cells in the CD group. Interestingly, in the HFD group, CtBP1-depleted PC3 cells developed significantly smaller tumors than animals inoculated with PC3 control cells (Fig. 3B). In addition, we could not detect tumor growth in 40% of mice injected with PC3.shCtBP1 cells and fed with a HFD, while all the animals of the other three groups developed tumors.

Remarkably, tumors from HFD-fed mice showed significantly reduced NAD+/NADH ratio which strongly support our hypothesis that HFD induces NADH levels (Fig. 3C). Moreover, estradiol levels in HFD xenografts were increased but no significant changes in intratumoral testosterone levels were observed (Supplementary Fig. S3A and S3B).

Histologic characterization revealed that all tumors were poorly differentiated adenocarcinoma (Fig. 3D). Moreover, CtBP1 depletion was confirmed in PC3.shCtBP1 xenografts by IHC and RT-qPCR compared with PC3.pGIPZ control tumors (Fig. 3D and Supplementary Fig. S3C).

Figure 2. HFD consumption for 16 weeks induced metabolic syndrome–like disease in mice. A, weekly average body weight from nu/nu mice fed with CD or HFD was plotted normalizing data to initial body weight. Box plots for (B) cholesterol and (C) total testosterone levels for CD- or HFD-fed mice are shown. Boxes represent the interquartile range, the horizontal line within each box represents the median, and the top and bottom whiskers represent the SD of one independent experiment. **, P < 0.01.
CtBP1 modulates cell adhesion, cell cycle, and steroid hormone response pathways

Because only HFD-fed animals inoculated with PC3.shCtBP1 cells showed tumor growth impairment, we next investigated the molecular pathways deregulated by CtBP1 depletion that might decrease tumor development in vivo. We performed a whole-genome expression array (Affymetrix HuGene 1.0 ST) from PC3.shCtBP1 and PC3.pGIPZ tumor xenografts growing in HFD-fed mice. Three independent samples for each condition were used. Our results showed 823 genes differentially expressed (fold-change > 1.5; P < 0.05) comparing PC3.pGIPZ with PC3.shCtBP1 xenografts from HFD-fed mice. Functional gene ontology analysis revealed that most of these genes correspond to metabolic processes (44.1%), cell communication (30.7%), and cell adhesion (11.7%) among other biologic functions (Fig. 4A).

Furthermore, Gene Set Enrichment Analysis (GSEA) revealed enhancement of genesets associated with cell-cycle progression and proliferation (Supplementary Table S1). In addition, PC3.shCtBP1 samples showed enrichment of genesets associated with cell adhesion, hormone metabolism, and BRCA1 targets gene regulation (Supplementary Table S2).

CtBP1 depletion in xenografts from HFD-fed mice induces E-cadherin–related pathways

GSEA further allowed the identification of a large number of genes associated with cell adhesion genesets (Supplementary Fig. S4A). Enrichment plot and heatmap from a selected cell adhesion geneset were shown. Genes listed alongside Figure 3. CtBP1 depletion decreases tumor growth in HFD-fed mice. Tumor growth curves from (A) CD or (B) HFD male nu/nu mice inoculated with PC3.pGIPZ or PC3.shCtBP1 cells after 12 weeks of diet. Each data point represents tumor volume average and SD from each group of animals. C, tumors removed were used to determine NAD⁺/NADH ratio. Plotted boxes represent the interquartile range, the horizontal line within each of the boxes represents the median, and the top and bottom whiskers represent the SD. *P < 0.05. D, H&E and CtBP1 IHC staining from tumor sections generated as xenografts in CD- or HFD-fed mice (×250 magnifications). HFD PC3.shCtBP1 tumors showed irregular and hyperchromatic nuclei with several necrobiosis and leukocyte foci.
 heatmap were upregulated in PC3.shCtBP1 compared with control PC3.pGIPZ tumors. Notably, a crucial cell adhesion gene over-represented in these genesets was CDH1 (E-cadherin; Fig. 4B and Supplementary Fig. S4A). We validated this finding by RT-qPCR from tumor xenografts using CDH1-specific primers and found that CDH1 was overexpressed in PC3.shCtBP1 tumors (Supplementary Fig. S4B). IHC CDH1 analysis showed that PC3.shCtBP1 xenografts exhibited moderate to high E-cadherin staining at single cells, mainly at the cellular membrane of necrobiosis foci, whereas in PC3.pGIPZ tumors, E-cadherin staining was low to moderate, diffused and located at single cells in necrobiosis foci (Fig. 4C).
CTBP1 depletion in xenografts from HFD-fed mice induces aromatase-related pathways

We found several steroid hormone metabolism-related genes over-represented and overexpressed in PC3.shCTBP1 xenografts compared with control PC3.pGIPZ as indicated in heatmap and enrichment plots in Fig. 4D and Supplementary Fig. S4C. From these analyses, we found that one of the genes that was over-represented in the genesets related to hormone biosynthesis and metabolism was CYP19A1 (aromatase enzyme), involved in the estradiol synthesis by conversion from testosterone. We validated this finding by RT-qPCR from tumor xenografts using specific primers for CYP19A1. As shown in Fig. 4E, CYP19A1 transcription was significantly induced in PC3.shCTBP1 compared with pGIPZ control.

These results are consistent with the metabolic syndrome–like disease because HFD-fed mice showed hormone imbalance. Nevertheless, the fact that CTBP1 depletion induces aromatase expression is a novel finding that reinforces the role of hormones and fat in tumor growth.

CTBP1 depletion in xenografts from HFD-fed mice decreases cyclin D1 expression

PC3.pGIPZ xenografts also showed a marked enrichment of genesets related to cell-cycle regulation and proliferation (Supplementary Table S1 and Supplementary Fig. S5). Genes in these categories included several cyclin proteins (CCND1, CCND2, and CCND3) and BRCA1, among others. We focused our studies particularly on CCND1 (cyclin D1). We validated the expression of this gene by RT-qPCR in tumors from HFD-fed animals and we found that CCND1 is significantly diminished in PC3.shCTBP1 xenografts compared with control (Fig. 5A). In addition, by IHC we found that PC3.pGIPZ tumors presented very high CCND1 immunoreactivity, whereas CTBP1 depletion completely abolished CCND1 staining (Fig. 5B).

CTBP1 and steroid hormones impair prostate tumor growth probably through BRCA1 regulation

It was previously reported that CTBP1 represses BRCA1 transcription in breast cancer cells (8). Here, we found by GSEA that PC3.shCTBP1 xenografts showed an enrichment of genes regulated by BRCA1 (Fig. 5C). Moreover, IHC analysis determined that CTBP1 depletion increased BRCA1-positive immunostaining compared with control xenografts from HFD-fed animals (Fig. 5D).

On the basis of several factors that might be involved in BRCA1 transcription regulation in prostate cancer, such as steroid hormones and CTBP1, we first determined BRCA1 transcription in the stable CTBP1 PC3 cell lines. We found that CTBP1 repressed BRCA1 transcription in vitro, whereas CTBP1 depletion highly increased BRCA1 expression compared with control PC3.pGIPZ cells (Fig. 6A). Then, we exposed three prostate cancer cell lines that differ in AR expression and androgen sensitivity to different testosterone concentrations. We found that BRCA1 mRNA levels significantly increased in PC3 cells exposed to testosterone, even after transfecting with the constitutive active AR vector (AR5; Fig. 6B). Similar results were found using 22Rv1 cells that express AR but are androgen insensitive (Supplementary Fig. S6A). However, testosterone decreased BRCA1 mRNA levels in LNCaP cells (AR+/− and androgen sensitive; Supplementary Fig. S6B).

Moreover, by ChIP we determined that CTBP1 was associated to BRCA1 proximal promoter region, and testosterone released CTBP1 from this region (Fig. 6C). Altogether these results indicate that BRCA1 transcription is regulated in prostate cancer cell lines by CTBP1 and testosterone.

It was previously reported that BRCA1 transcription is controlled by estradiol in breast cancer cell lines (8). To investigate whether BRCA1 transcription regulation in prostate cancer is due to testosterone or estrogen, as a consequence of testosterone conversion to estradiol by the aromatase enzyme, we assessed BRCA1 transcription in the presence of testosterone and an aromatase inhibitor (letrozole) that inhibits this conversion. We found that letrozole abolished BRCA1 induction by testosterone in both PC3 (Fig. 6D) and 22Rv1 (Supplementary Fig. S6C) cells; however, letrozole has no effect over BRCA1 transcription in LNCaP cells exposed to testosterone (Supplementary Fig. S6D).

In summary, these observations indicate that BRCA1 transcription regulation by testosterone in prostate cancer cells is modulated by aromatase-derived estrogen. Hence, CTBP1 and steroid hormone imbalance induced by the metabolic syndrome disease, govern BRCA1 transcription, which in turn, influences tumor suppression impeding prostate tumor growth.

Discussion

CTBP1 is a transcriptional corepressor that binds to histone modifiers and recruits repressive complexes to tumor suppressor promoters to inhibit their expression. It is becoming increasingly clear that dysregulation of CTBP1 transcriptional repression plays a crucial role in tumorigenesis (4, 8, 12). We present new evidence for CTBP1-mediated oncogenesis in prostate cancer. Our studies demonstrated that gene transcription regulation by CTBP1 provides an important molecular link among calorific intake, CTBP1 function, and tumor growth. We demonstrated that CTBP1 overexpression induces cell transformation and proliferation.

We developed a metabolic syndrome–like disease in nude mice adding high fat content to their diet to modulate CTBP1 activity in a reduced NAD+/NADH ratio cellular context. These mice showed a clear hormone imbalance, hypercholesterolemia, and histology alterations in their liver and kidneys. Surprisingly, CTBP1 knockdown delays tumor growth in these mice, suggesting that tumor growth in HFD-fed animals is CTBP1 dependent.

Several reports showed that HFD induced tumor growth in prostate cancer xenografts using AR+/− cell lines (22–25). Here, we found that HFD did not significantly influence tumor growth using the AR−/− cells (PC3) xenograft model. In addition, Wang and colleagues demonstrated that CTBP1 depletion decreased DU145 tumor growth in mice on normal chow diet (12). Here, CTBP1 depletion markedly
diminished tumor growth only in HFD-fed mice. We believe that intratumor hormone and CtBP1 levels, and probably AR-cell status are crucial "collaborating" factors that are involved in prostate cancer growth.

Takayama and colleagues recently demonstrated that CtBP1 regulates AR signaling (26), showing that CtBP1 exerts tumor-suppressive effects in AR<sup>+/+</sup> prostate cancer cells. In contrast, Wang and colleagues (12) described that CtBP1 is upregulated in metastatic prostate cancer and CtBP1 exerts growth stimulatory effects in tumor cells. We demonstrated here that testosterone downregulates CtBP1-mediated transcription targets in AR<sup>/−</sup>/cells; meanwhile in AR<sup>+/−</sup> cells, testosterone upregulates CtBP1 transcription targets. We believe that one of the reasons for the inconsistency in CtBP1 functions may be due to impairments in the AR status in the different tumor cell lines. Further experiments will be necessary to understand the mechanism of regulation mediated by CtBP1 in AR-positive or AR-negative cells.

Here, we found that CtBP1 represses BRCA1 transcription in prostate cancer. We showed for the first time that testosterone regulates BRCA1 transcription, probably via its conversion to estrogen by the aromatase enzyme. The fact that several genesets for steroid hormone secretion and biosynthesis were enriched in CtBP1-depleted tumors in addition to an enhance aromatase expression is a novel finding that reinforced hormone and fat role in tumor growth.

The role of NADH in regulating CtBP1 activity is also an important finding that increases researchers’ interest.
CtBP1 has been proposed to act as a metabolic sensor, responding to changes in NADH levels and modulating gene expression (7). The combination of high NADH content in tumor cells and high-fat-rich diets are factors that might affect carcinogenesis. Our work links reliably HFD and high NADH levels with CtBP1-dependent tumorigenesis. However, this novel molecular link might be further explored to understand the potential implications of these pathways in prostate cancer risk.

Expression array profiles from these xenografts showed that the major biologic processes affected by CtBP1 depletion are cell adhesion and communication, metabolism, and cell cycle. Finally, we were able to determine the potential role of CtBP1 as a gene transcriptional regulator and its implication in tumor development in prostate cancer. Both approaches, GSEA and in vivo xenograft model, allowed us to demonstrate that CtBP1 depletion dramatically decreased tumor growth and cell proliferation. Accordingly, Deng and colleagues generated CtBP1 transgenic mice (K5.CtBP1) that showed epidermis hyperproliferation by downregulation of p21 and BRCA1, and loss of E-cadherin expression (21).

We also found that CtBP1 depletion had implication in regulation of genes involved in cell adhesion such as CDH1. However, other important players were also deregulated by CtBP1 depletion and still have to be studied, such as integrin family (ITGB7, ITGAL, etc.), cadherins (CDH3, CDH15, etc.), and cell adhesion molecules (ICAM2, NCAM, CADM3, etc.). Furthermore, we found that several genes involved in cell communication and signaling also altered by CtBP1 depletion, probably having a great influence over prostate cancer tumor signaling and growth.

Obesity, type 2 diabetes and metabolic syndrome are placing an increasing burden on the healthcare systems. Many resources are currently being devoted to the identification of novel therapeutic targets that could alleviate or reverse the progress of these disorders. CtBP1 protein might be a critical target on these diseases. Recent studies have identified CtBP1 as a key transcriptional coregulator in adipose tissue (27). CtBP proteins interact with several different partners to regulate the development of both white and brown adipocytes. Manipulation of CtBP1 function may provide a mechanism by which energy storage in white adipose tissue can be limited and energy expenditure by brown adipose tissue can be increased.

Overall, our results reveal that CtBP1 governs crucial molecular pathways in prostate tumors in the presence of...
metabolic syndrome. Thus, it will be important to determine CtBP1 expression level status in the tumors from patients with metabolic syndrome to improve prognosis and disease management. In the future, it will be also important to correlate high fat consumption/weight gain/body fat/BMI with CtBP1 expression to better understand this role. Finally, CtBP1 pathway might help to identify new molecular candidates for a better prediction of the disease outcome.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: C.P. Moiola, P. De Luca, F. Zalazar, R. Meiss, P. Vach, M. Zerba, O. Pignataro. Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.P. Moiola, J. Cotignola, R. Meiss, P. Vach, M. Zerba, O. Pignataro, O. Mazza.

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Prostate Tumor Growth Is Impaired by CtBP1 Depletion in High-Fat Diet–Fed Mice

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