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Prohibitin Is a Cholesterol-Sensitive Regulator of Cell Cycle Transit

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ABSTRACT

Cholesterol is essential in establishing most functional animal cell membranes; cells cannot grow or proliferate in the absence of sufficient cholesterol. Consequently, almost every cell, tissue, and animal tightly regulates cholesterol homeostasis, including complex mechanisms of synthesis, transport, uptake, and disposition of cholesterol molecules. We hypothesize that cellular recognition of cholesterol insufficiency causes cell cycle arrest in order to avoid a catastrophic failure in membrane synthesis. Here, we demonstrate using unbiased proteomics and standard biochemistry that cholesterol insufficiency causes upregulation of prohibitin, an inhibitor of cell cycle progression, through activation of a cholesterol-responsive promoter element. We also demonstrate that prohibitin protects cells from apoptosis caused by cholesterol insufficiency. This is the first study tying cholesterol homeostasis to a specific cell cycle regulator that inhibits apoptosis. J. Cell. Biochem. 111: 1367–1374, 2010.

KEY WORDS: PROHIBITIN; CHOLESTEROL; CELL CYCLE; PROSTATE CANCER

Cholesterol, a steroid lipid, makes up almost one-third of the lipid content of the plasma membrane. The effects of cholesterol on membrane organization stem from its unique chemistry, where the combination of its stiff steroid structure and small head group affect its ability to pack with other lipids and proteins [Simons and Vaz, 2004]. Essentially, cholesterol orders the fluid membrane bilayer by limiting the gauche conformations that the acyl chains of phospholipids are able to assume. In animal cells, cholesterol content is a key regulator of membrane properties and its concentration in cell membranes is tightly regulated, even with wide variations in the external availability of cholesterol.

The ability of cells to maintain cholesterol homeostasis is in large part controlled by transcription factors that regulate many genes related to cholesterol synthesis as well as uptake. These sterol regulatory element (SRE) binding proteins (SREBPs) consist of the three related transcription factors, SREBP-1a, SREBP-1c, and SREBP-2 [Eberle et al., 2004; Bengoechea-Alonso and Ericsson, 2007]. Whereas SREBP-1c is primarily responsible for the transcription of fatty acid biosynthesis genes, including fatty acid synthase, SREBP-2 predominantly regulates mevalonic acid (cholesterol synthesis) pathway genes such as farnesyl diphosphate synthase (FDPS) and HMG-CoA reductase, the enzyme that catalyzes the rate-limiting step in cholesterol synthesis [Horton et al., 2003; Bengoechea-Alonso and Ericsson, 2007]. SREBP-1a controls transcription of a wide range of genes involved in cholesterol, fatty acid, and phospholipid synthesis [Eberle et al., 2004; Bengoechea-Alonso and Ericsson, 2007].

It has been known for several decades that cholesterol is critical for the proliferation of animal cells [Brown and Goldstein, 1974; Chen et al., 1974, 1975], and its synthesis is tightly synchronized to cell cycle progression [Chen et al., 1975]. Treatment of cells with statin drugs, which target HMG-CoA reductase and inhibit the synthesis of cholesterol and its upstream intermediates, causes cells to arrest in G1 [Chakrabarti and Engleman, 1991; Jakobisiak et al., 1991], but these studies cannot be interpreted as demonstrating a cholesterol dependence as they did not determine whether cholesterol or an upstream intermediate (e.g., geranylgeranyl pyrophosphate) was required. Further studies into the effects of statins on cell cycle progression have shown that lovastatin raises

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the levels of p21 and p27 cyclin-dependant kinase inhibitors [Poon et al., 1995; Gray-Bablin et al., 1997]. Consistent with these findings SREBP-1a has been shown to drive the expression of p21 [Inoue et al., 2005]. Taken together these studies demonstrate a mechanistic link between the cholesterol synthesis pathway and cell cycle inhibition. Studies aimed at determining the specific effect of cholesterol in cell cycle progression have demonstrated that limiting concentrations of cholesterol cause cells to growth arrest at the G2 stage, possibly through specific effects on $p34^{cdc2}$ [Martinez-Botas et al., 1999]. Whether the absence of cholesterol in these cases causes growth arrest due to insufficient materials for membrane synthesis, or a more specific regulatory role is not known, but certain phenomena suggest that a regulatory role is likely involved. For instance, in a number of different organisms (whether their main membrane sterol is cholesterol, ergosterol, or a phytosterol) absence of their main sterol leads to growth arrest, no matter how much a related, membrane-compatible sterol, for example, replacing cholesterol with ergosterol, is used. In these cases a small amount, insufficient for membrane synthesis, of native sterol is required to repair cell cycle progression [Clark and Bloch, 1959; Odriozola et al., 1978; Dahl et al., 1987; Whitaker and Nelson, 1988]. Moreover, blocking the cholesterol synthesis pathway downstream of HMG-CoA reductase, thus allowing for a more specific effect on cholesterol synthesis to be examined, demonstrated that cells become arrested at G2/M [Suarez et al., 2002; Fernandez et al., 2005]. This effect was in part explained by the ability of cholesterol, but not ergosterol, to induce cyclin B1 expression [Suarez et al., 2002]. How cholesterol may effect cyclin B expression is not known.

In exploring the proteome of prostate tumor cells for protein expression changes that are cholesterol sensitive, we uncovered one potential regulator of cell cycle progression, the protein prohibitin. Prohibitin is an evolutionarily conserved, ubiquitously expressed, 30 kDa protein that has been found in the cytoplasm, plasma membrane, nucleus, and mitochondria where it regulates cell cycle transit, has antiproliferative activity, regulates Ras-mediated mitogen-activated protein (MAP) kinase pathway activation, and appears to be essential for cell survival [Dell'Orco et al., 1996; Rajalingam et al., 2005; Gamble et al., 2007; Guo et al., 2007]. Here, we demonstrate: (1) that prohibitin expression increases when cholesterol levels are low; (2) that this regulation occurs on the transcriptional level; (3) that the prohibitin gene contains a promoter that responds to cholesterol insufficiency; (4) that prohibitin expression regulates cell responses to growth factors; and (5) that prohibitin expression protects cells from apoptosis due to insufficient cholesterol.

MATERIALS AND METHODS

CELL CULTURE

PC3 human prostate cancer cells (ATCC, Manassas, VA) were cultured in Dulbecco's Modified Eagle Medium, low glucose (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 5% CO_2 at 37°C. Cells were then treated with either normal medium (NM) supplemented with 10% FBS and 1% penicillin/streptomycin or with cholesterol depleted medium (CDM) supplemented with 10% lipoprotein-deficient fetal bovine serum (Sigma, St. Louis, MO), 1% penicillin/streptomycin, 50 μM mevalonate (Sigma), and 50 μM simvastatin (Teva Pharmaceutical, Israel) and were harvested after 48 h.

2D GEL ELECTROPHORESIS AND MASS SPECTROMETRY

PC3 cells were treated in NM or CDM for 48 h and then lysed as described [Solomon et al., 2000; Qi et al., 2009]. Protein (100 μg) from each lysate was desalted using Micro Bio-spin 6 columns (Bio-Rad), concentrated and resuspended in rehydration buffer. Isoelectric focusing, using IPG strips (Bio-Rad; 3–10 pH range) was carried out at 8,000 V, 50 mA/gel for 40,000 volt-hours total. Second dimension electrophoresis using 4–20% SDS–PAGE gradient gels was then performed, followed by staining, documentation, and protein excision. Reduced and alkylated proteins were then trypsin digested, reconstituted in 5% ACN, 5% formic acid, 90% H_2O, and subjected to LC/MS/MS by pressure-loading the samples onto a fused-silica microcapillary RP column packed with Magic C18 beads, with a 30 min gradient time. Peptides were ionized by electrospray and analyzed using a LTQ mass spectrometer (Thermo-Fisher). Raw files were converted using in-house software (Jethro), followed by searches using MASCOT (Matrix Science Inc.). Only peptides scoring $\geq$33 were considered.

EXPRESSION AND CLONING OF THE PROHIBITIN PROMOTER

Human PHB promoter (GenBank Accession No. DQ406856) [Theiss et al., 2007] was amplified by PCR using human chromosome 17 genomic DNA (clone RP11-1079K10; BacPac Resources, Children's Hospital Oakland Research Institute) as the template and using the sense primer: 5′-GCAACTCGAGGGAGAAACCCCGTCTCTAC-3′ (underline indicates a *Xho*I site) and antisense primer: 5′-GCAAAAGCTTCCTCACAAGTCGGACTCACGC-3′ (underline indicates a *Hin*dIII site). After sequence confirmation, the PHB promoter was subcloned into the PGL3 vector creating PGL3-PHB. PC3 cells were transfected with PGL3-PHB using Lipofectamine LTX Reagent (Invitrogen). After 6 h, medium was changed to MODS + 10% LPDS (LPDS) or LPDS supplemented with sterol (LPDS + CHO) (10 μg/ml cholesterol + 1 μg/ml of 25-hydroxycholesterol). After 36 h, cell lysates were prepared and luciferase activity was measured using the Luciferase Assay System (Promega) and luminometer (Promega). PGL3-LDLR promoter plasmid was used as a positive control and PGL3 basic vector plasmid used as the negative control.

RNA EXTRACTION AND REVERSE TRANSCRIPTION PCR

RNA was extracted using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. RNA was treated with DNaseI (Qiagen) and quantified by measuring the absorbance at 260 nm. cDNA was synthesized using 5 μg of RNA and Ready-To-Go You-Prime First-Strand Beads (GE/Amersham, Pittsburgh, PA). The PCR primer sequences were: 5′-GGAGGCGTGGTGAACTCTG-3′ (sense) and 5′-CTGGCACATTACGTGGTCGAG-3′ (antisense). Thermal cycles consisted of 40 cycles of 95°C for 30 s, 51°C for 30 s, 72°C for 1 min followed by 72°C for 10 min. GAPDH was used as an internal control.

REAL-TIME PCR ANALYSIS

Each reaction tube contained 1.5 μl of 100 μmol/L primer (a pair) + 15 μl SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) + 1 μl cDNA + 6 μl of DNase-free water. Amplifica-

tion and detection were performed using the ABI PRISM 7900HT sequence detection system (Applied Biosystems) with the following cycle profile: 45 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min. GAPDH was used as an internal control. Relative gene expression was analyzed using the 2-ΔΔCT method [Schmittgen et al., 2000].

IMMUNOBLOTTING

PC3 cell lysates were prepared as previously described [Solomon et al., 2000; Qi et al., 2009] and protein was measured using the BCA protein Assay (Thermo/Pierce, Waltham, MA). Equal amounts of lysate were subjected to SDS–PAGE and immunoblotting as previously described [Solomon et al., 2000; Qi et al., 2009]. Antibodies used were mouse anti-human PHB antibody (Abcam, Cambridge, MA) diluted 1:1,000 and mouse anti-human β-actin antibody (Sigma) diluted 1:1,000.

DELETION CONSTRUCTS

To determine the specific region for basal PHB promoter activity, we made a series of successive promoter truncations with six different sense primers (underlined nucleotides indicate an *Xho*I site):

Trun 1: 5′-GCAACTCGAGTGCACTCCAGCCTGGGCAA-3′ (−911/+138);
Trun 2: 5′-GCAACTCGAGGAGCCAAAGTTTAACGTGCGTT-3′ (−721/+138);
Trun 3: 5′-GCAACTCGAGCTGAGGACGTTTGCAGACAG-3′ (−508/+138);
Trun 4: 5′-GCAACTCGAGGGCTCTCCTTGCGGCAGTCTTA-3′ (−318/+138);
Trun 5: 5′-GCAACTCGAGGTAGTTCATCTGCTAAGAGCCG-3′ (−154/+138);
Trun 6: 5′-GCAACTCGAGAGGAGCTCATGCGCAGTATGTG-3′ (−36/+138);

and 5′-GCAAAAGCTTCCTCACAAGTCGGACTCACGC-3′ as antisense primer (underlined nucleotides indicate a *Hin*dIII site). The truncated PHB promoter constructs were created using the full-length PHB promoter as a template and performing PCR with the following cycle profile: denature the template at 94°C for 2 min, followed by 35 cycles at 94°C for 15 s, anneal at 55°C for 30 s and extend at 68°C for 1 min/kb followed by 72°C for 10 min. These truncated constructs were cloned into PGL3 vector, transfected, and tested for activity using the luciferase assay as described above.

MUTATION OF THE PUTATIVE REGION RESPONSIBLE FOR THE CHOLESTEROL SENSITIVITY OF PROHIBITIN

After narrowing down the region, we examined the prohibitin gene promoter for sequence homology to known SREs and identified the most likely SREs in this region (153/−35) as 5′-GGTTCTAAGC-3′ (−117/−108). Site-specific mutation was introduced into the wild-type −1054/+138 bp PHB promoter in PGL3 using the Quick Change Site-directed Mutagenesis Kit (Stratagene, San Diego, CA) and oligoes 5′-GCAGAACCAGGGTGACTCATATGAGCACCCTTCCCAGAAC-3′; 5′-GTTCTGGGAAGGGTGCTCATATGAGTCACCCTGGTTCTGC-3′.

PCR conditions were as follows: 95°C for 30 s, then 18 cycles of 95°C for 30 s, 55°C for 1 min and 68°C for 1 min/kb of plasmid length followed by elongation at 72°C for 10 min. Methylated DNA was digested with *Dpn*I for 3 h, and then introduced into *Escherichia coli*. DNA sequencing was used to confirm the mutation. Plasmid transfection and luciferase assay were as described above.

PROLIFERATION ASSAY

PC3 cells were plated onto 96 well round bottom plates (1.5×10^4) with MODS + 10% FBS. After 24 h, medium was changed to MODS(NM) or MODS(CDM) with and without either PDGF (20, 40 ng/ml) or EGF (20, 40 ng/ml) for 72 h. Cell proliferation was determined by using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay (Sigma) according to the manufacturer's instructions. Absorbance was measured at 570 nm using a kinetic microplate reader (Molecular Devices, Union City, CA).

RNA INTERFERENCE

PC3 cells were transfected with three PHB (HSS143281, HSS143282, and HSS143283) stealth select RNAi (Invitrogen). Stealth RNAi Hi GC (Invitrogen) was used as a negative control. Cells were transfected using Lipofectamine 2000 according to the manufacturer's instructions. After 12 h, medium was changed to MODS(CDM) and the transfectants incubated for an additional 24 h. Total RNA extraction and reverse transcription were done as described above. Real-time PCR analysis identified PHB-HSS143283 as the most efficient at reducing prohibitin expression.

APOPTOSIS ASSAY

PC3 cells were plated onto 6-well plates (3×10^5) with DMEM + 10% FBS. When 30–50% confluent, cells were transfected with RNAi oligo using Lipofectamine 2000. After 12 h, the medium was changed to MODS(NM) or MODS(CDM). After which 40 ng/ml PDGF or EGF was added to the medium and cells were incubated for 72 h. Percentage of apoptotic cells was determined by flow cytometry using the Annexin V FITC Apoptosis Detection Kit (Calbiochem) following the manufacturer's instructions.

STATISTICAL ANALYSIS

All results in the text and figures are presented as means ± SD. Statistical significance of the results was determined using the Student's *t*-test. $P < 0.05$ is considered significant.

RESULTS

Incubating PC3 prostate tumor cells in cholesterol depletion media (CDM; see Materials and Methods Section) causes them to growth arrest, a phenomenon similar to that reported for other cell types [Brown and Goldstein, 1974; Chen et al., 1974, 1975; Martinez-Botas et al., 1999]. In order to obtain some insight into the processes and proteins involved in this cholesterol-sensitive growth arrest, we treated the PC3 cells in normal media and CDM as described above, but in this instance subjected the cells to detergent lysis and 2D gel analysis (Fig. 1A). Based on the relative expression levels observable in these experiments, we chose to further analyze by mass spectrometry (MS) protein species that appeared to increase in expression after treatment with CDM. Equivalent areas of 2D gels

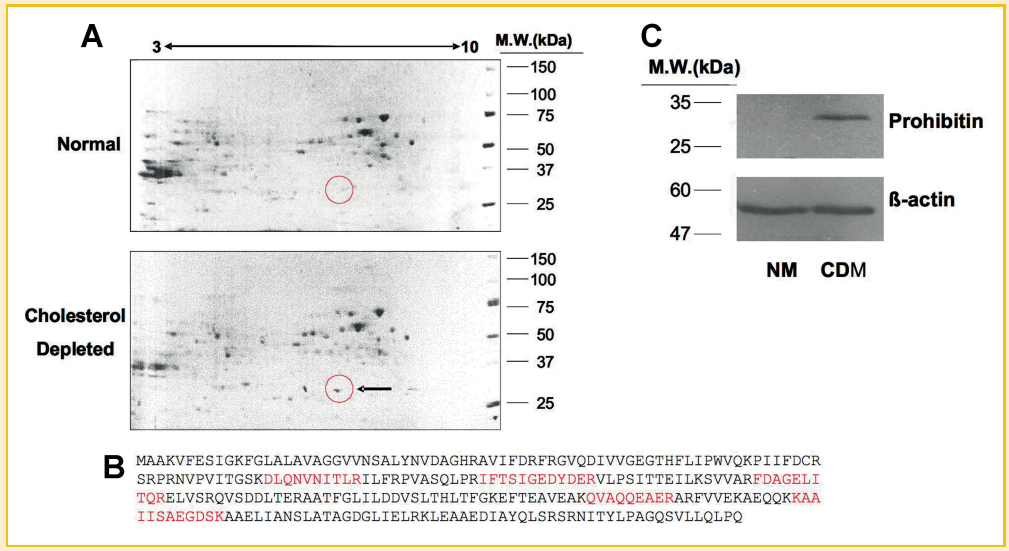

Fig. 1. Prohibitin protein expression is induced by cholesterol insufficiency. Cells were incubated in either normal media (NM) or cholesterol-depleted media (CDM) for 48 h and then subjected to: (A) 2D gel analysis and mass spectrometry (circle indicates area cut out for analysis, arrow indicates prohibitin). (B) Prohibitin sequence coverage from MS analysis of picked spot. Detected peptides are in red. (C) Immunoblot analysis using the indicted mAbs.

containing the resolved lysates of PC3 cells treated in normal media or CDM were excised, and subjected to MS analysis. From this analysis, we identified prohibitin as a 30 kDa species that increased in expression after CDM treatment (Fig. 1B). To confirm this finding, we again treated cells in normal media and CDM and performed immunoblot analysis using an anti-prohibitin mAb (Fig. 1C). The result confirmed that prohibitin protein expression increased after treatment in CDM.

To begin to develop an understanding of why prohibitin expression might be triggered by cholesterol insufficiency, we next determined that prohibitin mRNA increased $\approx 10\times$ in the presence of CDM (Fig. 2). This result suggested that prohibitin expression was likely to be regulated on the transcriptional level and that the prohibitin promoter may be sensitive to cholesterol insufficiency. Initially, we examined the prohibitin gene promoter for sequence homology to known SREs, identifying a number of putative sites but none of the analyzed sequences contained enough elements to define it as a SRE.

To examine the cholesterol sensitivity of the prohibitin promoter, we cloned the full-length promoter [Theiss et al., 2007] (GenBank Accession No. DQ406856.1) and tested its activation in response to cholesterol depletion using a luciferase reporter assay. The 1,192 bp clone containing the full-length promoter (-1054 to $+138$ with 1 being the transcriptional start site) was sensitive to cholesterol depletion when compared to the vector alone and more sensitive to cholesterol depletion than the LDL receptor promoter (Fig. 3A). Further analysis using this reporter system and sequentially truncated prohibitin promoter constructs allowed us to determine

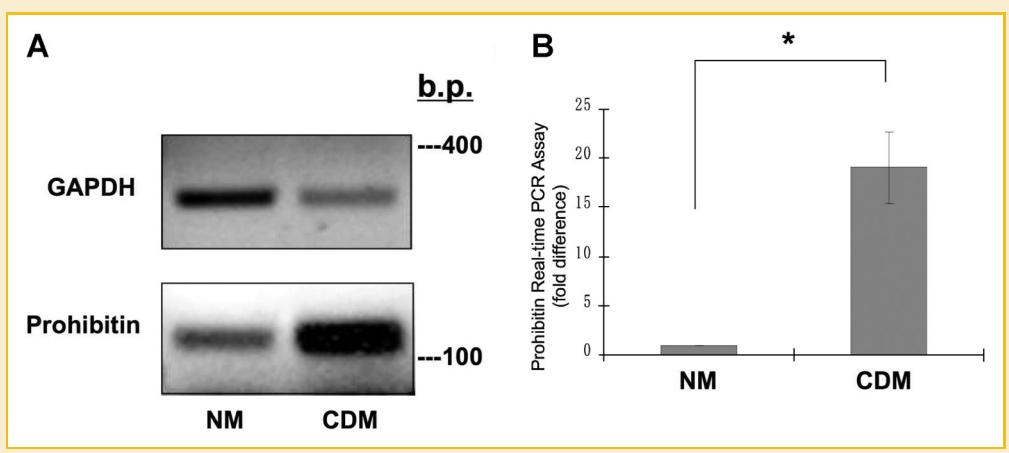

Fig. 2. Cholesterol insufficiency induces expression of PHB mRNA. PC3 cells were either cultured in NM or CDM for 48 h. Total RNA was then subjected to reverse transcription followed by PCR amplification using PHB or GAPDH specific primers as described in Materials and Methods Section. A: Electrophoretic analysis of RT-PCR. B: Real-time PCR analysis. Data are presented as average relative prohibitin mRNA expression ± SD of triplicate determinations ($^{*}P < 0.001$).

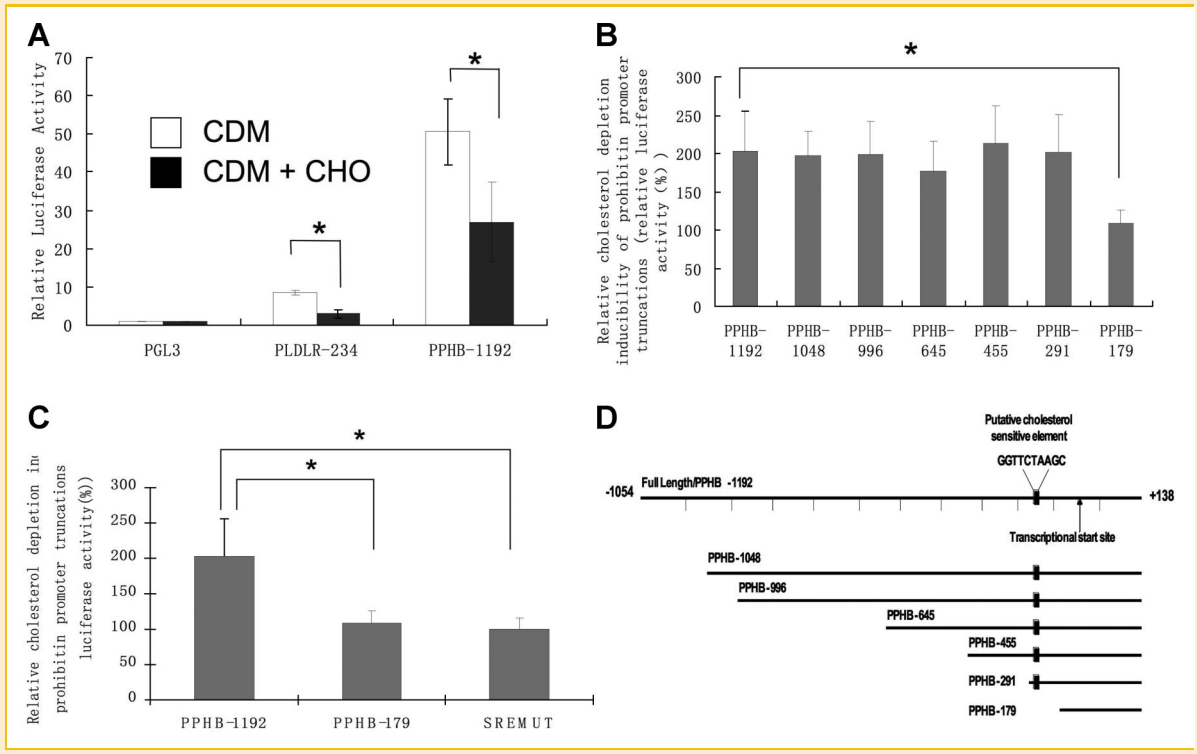

Fig. 3. Prohibitin promoter sensitivity to cholesterol insufficiency. A: CDM inducibility of the prohibitin and LDLR promoters is inhibited by cholesterol. PC3 cells transfected with PLDLR234LUC or PPHB1192LUC were treated with CDM ± cholesterol (CHO) (10 μg/ml cholesterol + 1 μg/ml of 25-hydroxycholesterol). Luciferase expression was assayed after 36 h. B: Localization of the PHB promoter cholesterol-sensitive element—truncation analysis. The full-length PHB promoter and sequential deletion promoter constructs (1048, 996, 645, 455, 291, and 179 bp) were transfected into PC3 cells. Six hours after transfection, cells were separately cultured with CDM ± cholesterol (CHO) and relative luciferase activity measured after 72 h. C: Localization of the PHB promoter cholesterol-sensitive element—mutational analysis. The full-length prohibitin promoter containing the putative cholesterol-sensitive element mutated by site-directed mutagenesis (SREMUT), the intact full-length promoter (PPHB-1192), and the truncated promoter construct (PPHB-179) were transfected into PC3 cells. Six hours after transfection, cells were cultured with CDM ± cholesterol (CHO) and the relative luciferase activity determined after 72 h. In parts A–C, the data are presented as the mean of relative luciferase activity ± SD of triplicate determinations ($^*P < 0.05$). D: Cartoon depicting scaled versions of the deletion constructs used in part B, with the putative cholesterol responsive element mutated in part C depicted.

that the responsive element was on the 3′ end of the promoter between −154 and −36 bp (Fig. 3B,D). Within this construct, we noted that a search of transcription factor binding sites indicated that the sequence GGTTCTAAGC (−118 to −109/reverse strand) had moderate homology to known SREs. Based on this and our truncation analysis, we replaced the putative cholesterol-responsive element with a mutated sequence in the full-length promoter luciferase reporter construct and tested its responsiveness to cholesterol depletion (Fig. 3C). These experiments revealed that the elimination of the putative cholesterol-responsive element significantly reduced the cholesterol sensitivity of the full-length promoter (Fig. 3B,C).

To determine the role of prohibitin in cell cycle regulation under conditions of insufficient cholesterol, we subjected PC3 cells to growth factor treatments (EGF and PDGF) in both normal media (cholesterol containing) and CDM. As anticipated, cells cultured in normal media with either PDGF or EGF proliferated to a greater extent than cells that were mock treated, while cells cultured in CDM with either PDGF or EGF not only failed to proliferate (cell numbers actually decreased), but also became apoptotic (Fig. 4).

To investigate what, if any, role prohibitin played in these growth factor responses, we tested the effect of RNAi-mediated knockdown of prohibitin expression (Fig. 4). We initially tested three RNAi constructs versus a control RNAi construct on their ability to inhibit prohibitin expression. RNAis #1 and #2 were effective at reducing prohibitin expression, with RNAi #1 proving to be the most efficient of the three and thus was used in subsequent experiments. When this RNAi was introduced versus a scrambled RNAi construct into PC3 cells, which were then cultured in normal media, proliferation in response to either PDGF (Fig. 4A) or EGF (Fig. 4B) was increased. In contrast, when cells were cultured in CDM, specific knockdown of prohibitin caused a decrease in cell number as well as a significant increase in apoptosis versus cells that were transfected with a control RNAi (Fig. 4C).

DISCUSSION

In this report, we demonstrate that prohibitin is a cholesterol-sensitive regulator of cellular proliferation. To support this finding, we demonstrate: (1) unbiased, discovery proteomics revealed that prohibitin, a known negative regulator of cell cycle progression, was upregulated by cholesterol depletion. (2) Immunoblot and qPCR analysis confirmed the unbiased result and suggested that prohibitin expression was regulated on the mRNA level. (3) Promoter analysis

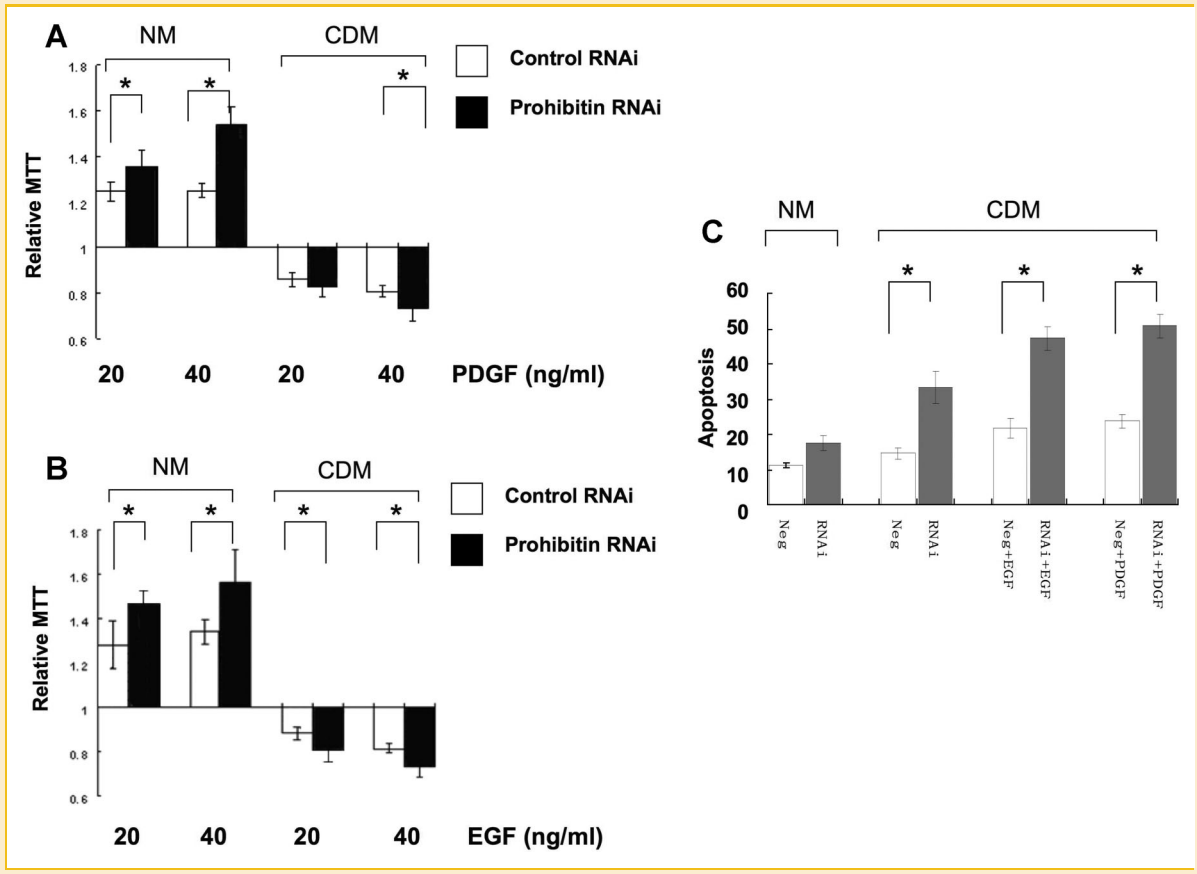

Fig. 4. Prohibitin expression reduces growth factor responses and protects cells from CDM-induced apoptosis. Six hours after PC3 cells were transfected with either a prohibitin RNAi or control RNAi construct the cells were cultured in either NM or CDM for 12 h. After which time either 20–40 ng/ml (see figure) PDGF (A) or EGF (B) was added to some cultures and the experiment continued for an additional 72 h. A,B: Proliferation. MTT was used to determine cell number as described in Materials and Methods Section. The data are presented as the mean of relative MTT assay ± SD of quadruple determinations ($^*P < 0.05$). C: Apoptosis. Apoptosis was measured as described in Materials and Methods Section using flow cytometry. The data are presented as the mean percent of apoptotic cells ± SD of triplicate determinations ($^*P < 0.05$).

demonstrated that the 3′ end of the prohibitin promoter contained a regulatory element that was cholesterol sensitive and that the sequence GAGGTTCTAAGC contained between DNA sequences −118 and −109 of the prohibitin gene is a probable cholesterol responsive element. (4) Finally, siRNA-mediated knockdown of prohibitin inhibited cells from growth arrest following cholesterol depletion resulting in an increase in apoptosis. Collectively, these results suggest that cellular sensing of cholesterol concentration regulates cell cycle progression and that prohibitin is one cholesterol-sensitive regulator of cell proliferation.

Prohibitin is a highly conserved protein with homologues in all eukaryotes, suggesting an important evolutionarily conserved function. Its precise biological role, though, remains unclear: prohibitin is found in the inner membrane of mitochondria in large assemblies where it acts as a molecular chaperone and exerts control over cellular proliferation as well as cristae morphogenesis [Merkwirth and Langer, 2009]; prohibitin is also found in the nucleus, where it associates with various proteins including nuclear corepressor (NCoR), histone deacetylase 1 (HDAC1), and retinoblastoma (Rb) leading to the functional repression of the E2F family of transcription factors [Wang et al., 2002a,b; Gamble et al., 2004]. Prohibitin has also been demonstrated to be localized at the plasma membrane, where it has surface exposure [Sharma and Qadri, 2004], and within cholesterol-rich lipid raft domains, some of which may be of mitochondrial origin [Kim et al., 2006; Staubach et al., 2009]. Here, we demonstrate that prohibitin regulates the growth factor responses of prostate tumor cells; within cells cultured in cholesterol containing media prohibitin acts to reduce proliferation in response to growth factor treatment; in cells cultured in cholesterol depletion media prohibitin acts to prevent growth factor-induced apoptosis. Interestingly, a very recent article reported in supplemental data that prohibitin mRNA increased 2.47-fold when Hela cells were cultured for 6 h under cholesterol-depleting conditions [Bartz et al., 2009].

SREBPs control the expression of genes related to the synthesis and uptake of fatty acids and cholesterol and are quite responsive to low levels of cholesterol through sterol sensing in the ER [Eberle et al., 2004]. Only recently SREBPs have shown to also regulate genes involved in cellular proliferation [Motallebipour et al., 2009], including host cell factor 1, filamin A, and ribosomal protein S9. SREBP1a has been demonstrated to activate the promoters of cdk inhibitors p16 and p21, and induce expression of p16, p21 as well as p27. The enforced expression of SREBP1a leads to inhibition of cdk2 and cdk4 through a mechanism that does not alter their expression

levels [Nakakuki et al., 2007]. Our results regarding prohibitin suggest that the element responsive to cholesterol insufficiency has sequence similarity to known SREs, thus suggesting SREBP-induced gene activation. The identified sequence also overlaps with binding sites for HSF, AP-1, and CREB, thus additional evidence will be required before we will be able to determine if prohibitin expression in response to cholesterol depletion is mediated by SREBP or other transcription factors.

Prior work has shown that cholesterol insufficiency leads to growth arrest [Brown and Goldstein, 1974; Chen et al., 1974, 1975; Martinez-Botas et al., 1999; Gamble et al., 2007]. Other studies have suggested that this is not simply a matter of insufficient material to synthesize functional membranes, but a more specific regulatory role for cholesterol [Clark and Bloch, 1959; Odriozola et al., 1978; Whitaker and Nelson, 1988]. In this report, we demonstrate that maintenance of cholesterol homeostasis functionally intersects with cell cycle regulation through the expression of prohibitin.

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