An oncogenic role for the multiple endocrine neoplasia type 1 gene in prostate cancer

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Abstract

Prostate cancer is the second leading cause of cancer related deaths in US men, largely because of metastasis, which is ultimately fatal. A better understanding of metastasis biology will lead to improved prognostication and therapeutics. We previously reported 11q13.1 gain was independently predictive of recurrence after radical prostatectomy. Multiple endocrine neoplasia I (MEN1) maps to this region of copy number gain in aggressive prostate tumors and was shown to be the only gene at this locus at increased expression in prostate cancer. Here, we demonstrate an oncogenic role for MEN1 in prostate cancer using a variety of independent assays.

Keywords

MEN1; metastasis; oncogene

Introduction

The multiple endocrine neoplasia (MEN) tumor suppressor gene, MEN1, maps to 11q13.1 and encodes the menin protein that underlies the syndrome for which it is named, MEN 1.¹⁻³ The MEN1 gene is predicted to be capable of encoding a vast repertoire of splice variants and possibly proteins. It is well-established that mutations in MEN1 cause MEN 1 in an autosomal dominant manner.⁴ Tumors in MEN1 cases arise through a two-hit mechanism consistent with it being a tumor suppressor gene.⁵ Not surprisingly, MEN1’s role in biology and pathology has been studied predominantly in the parathyroid gland, pituitary gland and pancreas, however its function remains largely enigmatic. Several studies have shown a role for menin in transcriptional modulation through interaction with histone deacetylases and histone methyltransferases.⁶⁻⁷ Clinical manifestations of MEN1 are the result of overproduction of hormones such as insulin, parathyroid hormone or growth hormone.⁸⁻⁹ With the exception of gastrinomas, most MEN1-associated tumors do not metastasize.

In a previous genome wide array comparative genomic hybridization (aCGH) study aimed at identification of DNA based biomarkers predictive of postoperative recurrence and metastasis,
we identified the \textit{MEN1} locus.\textsuperscript{10} Paradoxically, the narrow region centered on \textit{MEN1} is gained and not lost in aggressive prostate tumors. Further, in an exhaustive gene expression study all other candidates at the locus were excluded.\textsuperscript{10} Moreover, expression of \textit{MEN1} in a set of matched normal and prostate tumor pairs was higher in all tumors with 11q13.1 copy number gain, and in one case with normal \textit{MEN1} copy number.\textsuperscript{10} The latter case suggests a mechanism independent of copy number gain for deregulation of \textit{MEN1} gene expression in prostate tumors. It also provides compelling evidence the \textit{MEN1} gene is the primary target of amplification. Elevated \textit{MEN1} expression in prostate tumors has also been observed in studies using expression microarrays.\textsuperscript{11} Taken together the evidence seems to suggest \textit{MEN1} can act as an oncogene in prostate tumors. Here we present evidence demonstrating \textit{MEN1}’s role in promoting growth and attenuating apoptosis further supporting \textit{MEN1}’s role as an oncogene in cancer of the prostate.

\textbf{Materials and methods}

\textbf{Fluorescent \textit{in situ} hybridization to tissue microarray}

The CTD-2220I9 BAC clone from Invitrogen (Carlsbad, CA, USA) containing \textit{MEN1} was used to make a fluorescent \textit{in situ} hybridization (FISH) probe. The clone was streaked on chloramphenicol LB plates. Single colonies were isolated for 3 ml starter cultures. DNA was isolated by following the manufacturer’s protocol for the Plasmid Midi DNA kit (Qiagen, Valencia, CA, USA). The DNA was directly fluorescently labeled by following a modified random priming protocol. Briefly, two labeling reactions were set up that each contained 100 ng BAC DNA, 1 x Random Primers (Invitrogen BioPrime kit) and water such that the volume was 16 \(\mu\)l. The mixture was denatured at 99 °C for 15 min and then immediately put on ice. A total of 20 units of Klenow (Invitrogen BioPrime kit), 2 \(\mu\)l of FITC-dCTP (1 mM, PerkinElmer, Waltham, MA, USA) and 2 \(\mu\)l of a dNTP solution (2 mM dATP, dGTP and dTTP, and 0.5 mM dCTP) were added and then incubated O/N at 37 °C. The two labeling reactions were combined and ethanol precipitated in the presence of COT-1 (Human Hybloc; Applied Genetics, Melbourne, FL, USA). FISH studies were carried out in Dr Mark Rubin’s Laboratory. For detailed FISH procedure see Perner \textit{et al.}\textsuperscript{12} FISH signals were analyzed using digital imaging microscopy. At least 100 cells were attempted to assess per case. The tissue microarray (TMA) consisted of 30 metastatic prostate tumors from various organ sites obtained at autopsy.\textsuperscript{13}

\textbf{Immunohistochemistry to TMA}

Immunohistochemistry (IHC) was carried out with a polyclonal \textit{MEN1} antibody (generously donated by Dr Francis Collins and Dr Lee Burns of the NIH) at a 1:1500 dilution. Antigen retrieval was performed by heating the slides for 7 min in 10 mM citric acid buffer. The TMA consisted of cores from 48 patients with prostate cancer that were at high risk of recurrence at the time of surgery according to the Kattan nomogram. Each case had three or more representative cores.

IHC calls were made independently by two pathologists (SP, JS). Pathologist 1 (SP) made a positive call if \(\geq 5\%\) of the cells showed \textit{MEN1} staining. Pathologist 2 (JS) made a positive call if \(\geq 5\%\) of the cells showed staining with 2+ or 3+ staining. 2+staining is observed with a \(\times10\) and 3+ with a \(\times4\) objective. The call was further defined, by both pathologists, as occurring in the nucleus or the cytoplasm.

\textbf{Cell culture}

Prostate cancer cell lines including PC3, DU145, LNCaP, MDAPCA2A, and a breast cancer cell line BT474 were obtained from American Type Culture Collection (Manassas, VA, USA) and grown according to manufacturer’s protocols.
**MEN1 knockdown with siRNAs**

siRNAs targeting four different regions of the MEN1 coding sequence were obtained from Qiagen. DU145 cells were transfected with siRNAs targeting MEN1, a scrambled siRNA (nonhomologous to any known human cDNA sequence) or transfection reagent alone in serum-free conditions, 24 h after seeding. Each of serum-free media (25 μl) with 0.75 μl of HiPerfect (Qiagen) and a 5 nM solution of siRNA were prepared separately, incubated for 5 min at room temperature (RT) and then combined. The liposome–siRNA complexes were then allowed to form, by further incubation for 15 min at RT. This 50 μl transfection mixture was then added to existing media on the cells and left to incubate at 37 °C for at least 24 h before desired treatment. Volumes listed are for a 96-well format required for the ACEA experiments described below, and were adjusted accordingly for the six well assays in the fluorescence-activated cell sorting (FACS) and reverse transcription (RT)–PCR experiments.

**ACEA**

The ACEA (San Diego, CA, USA) RT-CES ×16 system was used to monitor the cell growth index in MEN1 knocked down cells. ACEA’s technology measures the electronic impedance of sensor electrodes integrated on the bottom of microtiter plates. Cells are plated onto these special wells, and grown in a 37 °C incubator under 5% CO₂. The electrical impedance is then used to derive a cell index, which when continually monitored, gives a real time representation of the growth characteristics of the cells in the well.

Background impedance measurements were made in each well with media. A total of 1000 DU145 cells were then seeded, in a total of 150 μl media, per well. At 24 h later, transfections were carried out in serum-free media. The cell index was then continuously monitored, during serum starvation as well as post-rescue with serum media, for the various experimental conditions. Serum starvation (48 h) was found to be the optimal time period to show arrest of cell growth in MEN1 siRNA-transfected cells whereas maintaining viable cells. In addition, cell counts were confirmed manually (data not shown).

**Fluorescence-activated cell sorting (Annexin V and propidium iodide)**

Cells were seeded in duplicate six-well plates and transfected 24 h later in serum-free conditions. At 48 h post-serum starvation, cells were rescued with serum for a further 48 h before being collected by trypsinization. Cells grown in serum media, nontransfected serum starved cells, mock transfected (transfection agent only) serum starved cells, and scrambled siRNA-transfected serum starved cells were controls. Culture medium was discarded after cells were pelleted by centrifugation for 5 min. Cells were dissociated and suspended in 5 ml phosphate-buffered saline (PBS). A cell count was performed for every sample and equal number of cells (approximately 10⁶) were retained in 500 μl PBS. The cells were then fixed in 5 ml of 70% ethanol for 30 min. Cells were spun down and ethanol removed. The cell pellet was suspended in 5 ml PBS and centrifuged again. Propidium iodide (PI)/Triton X-100 (1 ml; Sigma, St Louis, MO, USA; 1 mg/ml PI in 0.1% Triton X-100) staining solution with RNaseA was added to stain the cells. Flow cytometry was performed after 15 min of staining at RT. Forward scatter and side scatter (FSC/SSC), respectively, and fluorescence 2 (FL2) were measured by FACS (FACS Calibur System; Becton Dickinson, San Jose, CA, USA) and analytical software (Treestar, Ashland, OR, USA; FlowJo) was used to gate single cells based on light scatter and analyze the resulting cell cycle distribution patterns using peak deconvolution (Deen-Jett, Watson).

Similarly, cells were also analyzed for apoptosis using Annexin staining posttransfection. Cells were isolated by trypsinization, washed in PBS then suspended in 100 μl of 1 × Annexin-binding buffer before staining with Annexin V (Molecular Probes-Invitrogen), according to manufacturer’s instructions. By FACS (Becton Dickinson FACS Calibur System), after FSC/
SSC gating was used to discriminate against debris and doublets, changes in live, dead and apoptotic subpopulation fractions were determined against negative treatment controls.

**MTS**

Cells were set up for transfection and treatment, as above, but in 96-well plates and in triplicates. At 48 h post-serum starvation, Promega’s (Madison, WI, USA) CellTiter 96 Aqueous One Solution Cell Proliferation assay was used according to manufacturer’s instructions to quantify NADH/NADPH levels in cells. 3-4,5-Dime-thylthiazol-2-yl)-5-3-carboxymethoxyphenyl)-2-4-sulfo-phenyl)-2H-tetrazoliun, inner salt (MTS) reduction was quantified by recording absorbance at 490 nm in a Wallac–PerkinElmer Victor2 1420 Multilabel counter.

**Quantitative RT–PCR**

RNA for the MEN1 siRNA knocked down DU145 cells and corresponding controls were isolated using Trizol (Invitrogen) in accordance with the manufacturer’s protocol and deoxyribonuclease (DNase) treated. cDNA was made using iScript First Strand Synthesis kit (BioRad, Hercules, CA, USA).

TaqMan assays to confirm MEN1 knockdown in the siRNA experiments were carried out by the UCSF Genome Core. TaqMan to confirm ABI expression array results for DU145’s MEN1 siRNA experiments were carried out by Dr David Ginzinger (LinkageBio, San Francisco, CA, USA). All TaqMan assays were carried out according to ABI protocols. TaqMan primer-probe sets were purchased as Assays on Demand kits through ABI (Foster City, CA, USA). All gene expression results were calculated relative to the expression levels of the housekeeping gene BGUS.

**ABI expression arrays**

http://bioconductor.org/packages/1.9/bioc/html/ABArray.html). Probe signal intensities across microarrays were normalized using the quantile method. Features with signal/noise values ≥3 and quality flag values <5000 were considered detected and were compared by t-test. The resulting gene lists with P-value <0.05 were then classified using the PANTHER database (http://www.pantherdb.org).

**Results**

**Fluorescence in situ hybridization to a tissue microarray**

Fluorescence in situ hybridization was carried out to further investigate copy number gain of the MEN1 locus in metastatic prostate cancer. FISH to a TMA containing cores from 30 metastatic prostate tumors was performed. The detailed results are reported in Supplementary Table S1. Two cases were excluded because of lack of tissue. Out of 28 readable cases, 26 had tissue from multiple metastatic sites. Defining gain of 11q13.1 as ≥10% of cells having ≥3 signals in at least one core for a patient, 5 out of 28 (18%) cases were positive. However, if the threshold was defined as ≥5% of cells having ≥3 signals in at least one patient core, 11 out of 28 cases (39%) were positive. Using the 5% threshold, 8 out of the 11 (73%) cases positive for 11q13.1 gain were positive in more than one metastatic site per patient. Figure 1a shows an example of increased copy number at the 11q13.1 locus in soft tissue and lung metastases from one patient. The TMA also included control cores of benign prostate tissue (N = 2), benign hyperplasia (N = 2) and prostatic intraepithelial neoplasia (N = 1) and normal breast tissue (N = 1), none of which were positive for MEN1 by FISH.
**Immunohistochemistry to a tissue microarray**

To investigate menin levels in prostate cancer, IHC was carried out with an MEN1 antibody on a TMA comprised of cores from 48 patients with prostate cancer that were at high risk for recurrence at the time of surgery as defined by the Kattan nomogram. The result details are tabulated in Supplementary Table S2. Of these cases, 36 had at least two cores that were able to be evaluated by two pathologists. There was high concordance in their calls with only three disagreements calls. One was a benign case being classified as either staining positive or negative. The other two disagreements questioned whether the tissue was benign or PIN, but both called the staining positive. Five of the thirty-six (14%) tumor cases stained positive for MEN1. The staining was detected in the nuclei of the tumor cells (Figure 1b). There was benign tissue available for a subset of the cases. Five of the thirty-six cases (14%) stained positive for MEN1 in the benign prostate glands. Interestingly, the staining in these cases was detected in the cytoplasm (Figure 1b).

**MEN1 expression database query**

A meta-analysis was conducted to evaluate MEN1 expression in published and publicly available prostate cancer studies. The Oncomine database (http://www.oncomine.org) was queried for studies that identified MEN1 expression in prostate tissue. Studies that contained cancer tissue and showed differential expression with a $P$-value $\leq 0.05$ are shown in Figure 2. In general, the trend for MEN1 expression in these studies was that MEN1 expression increased in primary and metastatic tumors as compared to benign tissue.

**MEN1 expression levels in cell lines**

RNA was extracted from various prostate cell lines (PC3, DU145, LNCaP, MDAPCA2A) to quantify MEN1 expression levels and to determine their suitability for in vitro knockdown experiments. BT474, a breast cancer cell line was included in the evaluation as well as lung cDNA as it is known to express low levels of MEN1. All of the cell lines expressed MEN1. Supplementary Figure S1 shows the TaqMan results for MEN1 in the five cell lines and the control. MDAPCA2A and LNCaP are semi-adherent cell lines and were not pursued for phenotypic studies.

**MEN1 siRNA knockdown experiments in DU145**

The in vitro ability of MEN1 to modulate cellular phenotypes such as growth was measured using RNAi. PC3, which is derived from a bone metastasis, did not respond to MEN1 siRNA knockdown experiments. However, DU145, which is derived from a brain metastasis did show a knockdown phenotype and was therefore utilized in all subsequent experiments. Cell growth was monitored in real time with the ACEA Biosciences RT-CES System, a cell-based assay that indirectly monitors the cell growth index by measuring electrical impedance across sensor electrodes integrated on the bottom of microtiter plates. Four independent siRNAs targeting different regions of the MEN1 transcript were evaluated. All appropriate controls were employed including scrambled siRNAs. Silencing of MEN1 suppressed cell growth in DU145 cells approximately 30% relative to controls (Figure 3a). TaqMan was used to confirm attenuation of MEN1 expression in the siRNA treated cells (Figure 3b). The siRNAs lowered the abundance of MEN1 mRNA by about 60%.

The MTS assay, a proliferation assay that is directly proportional to the number of live cells in culture, was used as an independent measure of proliferation in siRNA treated DU145 cells. These results support that silencing of MEN1 results in suppressed cell growth. Cell numbers were approximately 15% lower in MEN1 siRNA treated cells when compared to controls 48 h posttransfection, in serum starved conditions (Figure 3c).
To investigate the mechanism by which silencing MEN1 suppresses cellular proliferation, PI staining followed by FACS was used to quantify the distribution of cells in G1, S and G2/M phase in the different transfected cells (Figure 4a). MEN1 suppression resulted in an increase in the number of cells in S and G2 relative to the controls. Annexin staining increased in cells where MEN1 expression was silenced (Figure 4b). One can infer from these results that MEN1 depletion can increase apoptosis and cell cycle arrest in DU145 cells treated with MEN1 siRNA.

Expression arrays

To elucidate putative pathways perturbed by aberrant MEN1 expression in prostate tumors, we undertook expression microarray studies with RNA from the siRNA experiments. RNA from DU145 cells with confirmed MEN1 silencing (siRNA M4) was compared to control RNAs (every set in triplicate). Analysis was restricted to genes with expression changes with P-values < 0.05. Supplementary Table S3 lists the biological function categories that were overrepresented and underrepresented for the MEN1 siRNA expression studies. The expression of selected genes was then validated using TaqMan with MEN1 as a control. Figure 5a shows the results for integrin-β1 (ITGβ1), caspase 8 (CASP8) and p53 effector related to PMP22 (PERP). Figure 5b shows the validation of these genes using quantitative RT–PCR.

Discussion

In previous work using aCGH, we determined approximately 25% of primary prostate tumors that progressed to metastasis had gain at the MEN1 locus.10 Now using FISH on metastatic tumors we have detected gain at MEN1 in 18–39% of metastatic tumors depending on the threshold used. The metastases used to construct the TMA come from a rapid autopsy program and for most autopsies multiple organ sites are represented on the TMA. This allowed us to investigate heterogeneity for MEN1 gain across organ sites within individual cases. The majority of cases showed there was uniformity across organ sites. This may suggest a clonal origin for prostate cancer metastases and that MEN1 is at least associated with aggressive prostate cancer. The possibility that MEN1 may be associated with aggressive prostate cancer is supported by earlier work where gain at the MEN1 locus was found to be an independent predictor of disease recurrence following radical prostatectomy.10 Thus, it is possible that in prostate cancer MEN1 is a positive regulator of aggressive disease.

If increased MEN1 copy number is causally related to aggressive disease, then an increase in MEN1 gene expression would be expected in the corresponding tumor. Indeed, such an increase in expression in primary tumors has been reported.11 Further, a meta-analysis of public MEN1 gene expression data in prostate cancer reveals a trend toward increased MEN1 expression in prostate tumors and metastases compared to normal tissue in several different studies.18–22 The collective evidence suggests that MEN1 can act as an oncogene. If the MEN1 gene is acting as an oncogene then it should confer a phenotype consistent with that function. To test this, MEN1 transcripts were silenced using siRNA and cell proliferation monitored in real time using the ACEA RT-CES system. In multiple independent experiments, silencing of MEN1 in a prostate cancer cell line was accompanied by decreased cell proliferation and increased apoptosis. According to the manufacturer, curve shapes generated by the ACEA RT-CES system are indicative of specific cellular responses. These curves suggest a cytostatic response, which also was confirmed by manual cell counting. Furthermore, these results, a decrease in cell proliferation and an increase in apoptosis, were reproduced using the MTS assay and FACS analysis. These experiments provide the first evidence that MEN1 can act as an oncogene in vitro in the proper cellular context.

The siRNA experiments demonstrated that down-regulation of MEN1 enhances apoptosis. Therefore, we conducted expression microarray studies to identify downstream effectors of...
Menin. The expression array studies identified genes co-regulated with MEN1. Two genes, PERP and CASP8, can induce apoptosis and were observed to have increased expression when MEN1 is suppressed. Therefore, when MEN1 is over expressed in prostate tumors these positive effectors may be down-regulated, attenuating apoptosis. The CASP8 finding is in contradiction to what has been reported when MEN1 acts as a tumor suppressor. In that context, menin suppresses tumorigenesis partly by upregulating CASP8 expression. Similarly, ITGβ1’s expression increases when MEN1 expression is decreased. Therefore, when MEN1 dosage is increased, ITGβ1’s expression is decreased. Integrins are involved in cellular adhesion and proliferation and their deregulation can facilitate metastasis. This suggests a link for MEN1 in promoting prostate cancer metastasis.

Menin shows no homology to any known protein. Other than two nuclear localization signals, menin has no identifiable functional motifs. IHC studies by Guru et al. of interphase somatic cells showed that the menin protein is located in the nucleus. In an effort to elucidate the role of MEN1 in prostate cancer, we performed IHC studies. In benign prostate glands adjacent to tumor cells, IHC staining revealed menin localized to the cytoplasm although nuclear localization was observed in tumor cells. The gene’s two functional nuclear localization signals are consistent with our observation that the menin protein can localize to either the cytoplasm or nucleus. Interestingly, both normal adjacent prostate tissue and tumor can also stain negatively for menin.

There are many observations of MEN1 that imply molecular and biological complexity. First is not only can there be multiple foci per affected organ in MEN, but also that many different endocrine organs can be affected simultaneously. Second, several alternative splicing patterns exist for MEN1, suggesting the potential for multiple protein isoforms. Whereas most MEN1 tumors are benign, a subset are malignant. Interestingly, MEN1 patients who develop gastrinomas possess metastases at diagnosis, with 15% progressing to an aggressive malignancy. Even the traditional role of MEN1 as a tumor suppressor has been shown to be complex. MEN1 can act as a transcriptional activator, to maintain the expression of growth regulatory genes, and a transcriptional corepressor, negatively regulating growth promoting genes. Although MEN1 has historically been thought to act as a tumor suppressor, menin is considered an oncogene in mixed-lineage leukemia (MLL). Yokoyama et al. elegantly demonstrated that the physical interaction between the oncogenic MLL fusion protein and menin was required for the initiation of MLL-mediated leukemogenesis. Therefore, MEN1 can serve as an oncogenic cofactor in MLL. Finally, Karnik et al. also demonstrated that MEN1 is regulated by prolactin and progesterone raising the possibility that defects in signaling pathways regulated by steroid hormones might be linked to phenotypes associated with MEN1 inactivation. Conversely, it is conceivable that increased expression of MEN1 might play a role in hormone driven cancers, such as prostate cancer, as suggested here. Further studies will need to be performed to further elucidate the mechanism for MEN1’s oncogenic potential as well as clarify the role of co-regulatory genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr Nora Navone for generously donating the MDAPCA cells, Dr Francis Collins and Dr Lee Burns for kindly providing the MEN1 antibody, Bill Hyun and the UCSF Laboratory for cell analysis for assistance with FACS analysis, Applied Biosystems for supplying the reagents for the expression experiments, Christopher LaFargue for excellent technical assistance with the IHC experiments, and Dr Pankaj Kapahi, Jihad Skaf and Gregory Kronmal for reviewing the article. This work was supported by an RO1 from the National Institutes of Health (CA115484).
References


Figure 1.
Fluorescence *in situ* hybridization (FISH) and immunohistochemistry (IHC) examples. (a) FISH validation of 11q13.1 copy number gain in soft tissue (left) and lung (right) metastases from the same patient. Green signals represent the probe spanning the multiple endocrine neoplasia I (*MEN1*) locus, red signals represent the reference probe. (b) Representative IHC staining demonstrating *MEN1* expression in the cytoplasm of benign prostate tissue (left) and nuclear *MEN1* expression in a tumor sample (right) from different patients.
Figure 2.
Results of Oncomine database query for multiple endocrine neoplasia I expression in prostate cancer (differential expression $P<0.05$). The shades of gray within an analysis are to help distinguish each tissue type represented. The corresponding table legend describes each analysis.

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*Prostate Cancer Prostatic Dis. Author manuscript; available in PMC 2010 March 23.*
Figure 3.
Phenotypic effects of multiple endocrine neoplasia I (MEN1) siRNA silencing. (a) *In vitro* cell proliferation assay using an ACEA instrument showing decreased cell proliferation of DU145 cells transfected with four different MEN1 siRNAs (M1-4). Transfections were carried out in serum starved conditions; cells were rescued and maintained in serum media subsequently for 48 h. Controls include nontransfected (SFM), transfection reagent alone (HP) and scrambled siRNAs (AF, S5). (b) shows corresponding TaqMan data for the same samples confirming knockdown of MEN1 expression in cells with the siRNA, 72 h posttransfection. (c) DU145 cells were transfected in serum-free conditions and then rescued with serum media, 48 h later. Cell viability was quantified using the MTS assay, after 72 h of rescue. Samples include DU145
cells transfected with three different MEN1 siRNAs (M1, M3 and M4). Controls include nontransfected (SFM), transfection reagent alone (NC) and scramble siRNA (AF). Doxorubicin treated DU145 cells were included as an additional control (Dox500).
Figure 4. Fluorescence-activated cell sorter (FACS) analysis. (a) Cell cycle distribution of multiple endocrine neoplasia I (MEN1) siRNA treated DU145 cells. Transfected cells were analyzed by FACS to ascertain the effects of MEN1 knockdown on the cell cycle. DU145 cells were transfected and starved in serum-free media for 48 h before rescue with serum–media. 48 h later, cells were fixed in ethanol and stained with PI. Samples include DU145 cells transfected with 2 different MEN1 siRNAs (M1 and M4). Controls include DU145 cells (REG), nontransfected (SFM), transfection reagent alone (NC) and scrambled siRNA (AF). Light gray bars represent cells in G1 phase, dark gray bars represent S phase, and G2 phase cells are represented by white bars. Each sample was run in duplicate and the average is shown. (b) Transfected cells were analyzed by FACS to study the effects of MEN1 silencing on the induction of apoptosis. DU145 cells were transfected and starved in serum-free media for 48 h before rescue with serum media. After 48 h, cells were stained with Annexin to track phosphatidyl serine flipping in cell membranes that is a hallmark of early apoptosis. Samples include DU145 cells transfected with two different MEN1 siRNAs (M1 and M4). Controls include DU145 cells (Reg), nontransfected (SFM), transfection reagent alone (NC) and scramble siRNA (AF). Two different concentrations of doxorubicin treated DU145 cells were included as additional controls. Each sample was run in duplicate and the average is shown.
Figure 5.

(a) DU145 expression array results of selected genes. The gene and the corresponding expression array fold change are listed. Fold change is calculated as the ratio of the expression in multiple endocrine neoplasia I (MEN1) knocked down DU145 cells compared to the controls (no siRNA and scramble combined). (b) TaqMan validation of DU145 expression array results. Every gene’s expression was measured relative to that of the housekeeping gene BGUS. TaqMan results are shown for ITGβ1 (light gray), CASP8 (dark gray) and PERP. Each result is plotted as the percent expression relative to untreated DU145. Reg represents DU145 cells without siRNA, scramble are DU145 cells that were treated with siRNAs that share no homology to known cDNAs, MEN1 siRNAs are the DU145 cells treated with siRNA. Each bar represents two biological replicates that were each run in triplicate before averaging the results.