Golgi Protein GOLM1 Is a Tissue and Urine Biomarker of Prostate Cancer

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ABSTRACT

Prostate cancer is the most common type of tumor found in American men and is the second leading cause of cancer death in males. To identify biomarkers that distinguish prostate cancer from normal, we compared multiple gene expression profiling studies. Through meta-analysis of expression array data from multiple prostate cancer studies, we identified GOLM1 (Golgi membrane protein 1, Golm 1) as consistently up-regulated in clinically localized prostate cancer. This observation was confirmed by reverse transcription–polymerase chain reaction (RT-PCR) and validated at the protein level by immunoblot assay and immunohistochemistry. Prostate epithelial cells were identified as the cellular source of GOLM1 expression using laser capture microdissection. Immunohistochemical staining localized the GOLM1 signal to the subapical cytoplasmic region, typical of a Golgi distribution. Surprisingly,
GOLM1 immunoreactivity was detected in the supernatants of prostate cell lines and in the urine of patients with prostate cancer. The mechanism by which intact GOLM1 might be released from cells has not yet been elucidated. GOLM1 transcript levels were measured in urine sediments using quantitative PCR on a cohort of patients presenting for biopsy or radical prostatectomy. We found that urinary GOLM1 mRNA levels were a significant predictor of prostate cancer. Further, GOLM1 outperformed serum prostate-specific antigen (PSA) in detecting prostate cancer. The area under the receiver-operating characteristic curve was 0.622 for GOLM1 \( (P = .0009) \) versus 0.495 for serum PSA \( (P = .902) \). Our data indicating the up-regulation of GOLM1 expression and its appearance in patients’ urine suggest GOLM1 as a potential novel biomarker for clinically localized prostate cancer.

**Introduction**

Prostate cancer is the most commonly diagnosed malignancy and is a leading cause of cancer-related death in the Western male population \([1,2]\). Early diagnosis is critical for the effective treatment of malignant tumors. High-throughput technologies such as DNA and protein microarray have enabled the identification of genes and their corresponding proteins that are differentially regulated in malignant conditions \([3,4]\). These high-throughput studies have offered researchers a better understanding of the disease and the molecular circuitries that are dysregulated in cancer. Our previous DNA microarray studies using prostate cancer tissue RNA have identified multiple genes, including alpha methylacyl coenzyme A racemase \((AMACR)\), Enhancer of Zeste Homolog 2 \((EZH2)\), tumor protein \(TPD52\), and \(ERG\), as dysregulated in localized and metastatic prostate cancer \([5–8]\). Many of these findings provided new insights into the biology of prostate carcinogenesis and have identified the next generation of candidate biomarkers and potential therapeutic targets \([9–12]\). In the case of \(AMACR\), a peroxisomal fatty acid–metabolizing enzyme, the up-regulated protein was detectable in patients’ urine, which indicated its potential use for noninvasive diagnostic studies \([13]\). Furthermore, our studies indicated an immune response to \(AMACR\) in prostate cancer patients and autoantibodies directed against \(AMACR\) that could be detected in the patient’s serum \([14]\).

\(GOLM1\) (Golm 1, NM_016548) is a resident cis-Golgi membrane protein of unknown function. The first evidence of its up-regulation was shown in the hepatocytes of patients with acute and chronic forms of hepatitis and hepatocellular cancer \([15]\). GOLM1 has a single N-terminal transmembrane domain and an extensive C-terminal, coiled-coil domain that faces the luminal surface of the Golgi apparatus. N-terminal cleavage by a furin proprotein convertase resulted in the release of the C-terminal ectodomain and its appearance in serum \([16]\). The cleaved form of GOLM1 was detectable in the serum of patients with hepatocellular cancer, a finding that may have diagnostic value \([17]\). Initial gene expression array studies in our laboratory, and by others \([3,18]\) suggested increased expression levels of GOLM1 mRNA in prostate cancer tissues. We subsequently detected GOLM1 transcripts in patient urine samples. By multiplexing with other urine markers, we demonstrated that GOLM1 mRNA levels can serve as significant predictors of prostate cancer \([19]\). Our study confirms the epithelial cell–specific expression of GOLM1 in prostate cancer tissues. Furthermore, we show that the GOLM1 protein is released from prostate cell lines \textit{in vitro} and is detectable in the urine of patients with established prostate cancer. The secretion from cell lines could be inhibited by treating cells with the protein transport inhibitor brefeldin A \([20,21]\). Our observation suggests that GOLM1 has potential as a noninvasive biomarker of localized prostate cancer.

**Materials and Methods**

**Quantitative Real-time Polymerase Chain Reaction**

To validate GOLM1 overexpression observed in multiple gene expression profiling studies, we performed quantitative real-time polymerase chain reaction (qPCR) for GOLM1 expression using SYBR green \([6,22]\). Briefly, cDNA was made with the total RNA isolated from 11 benign prostatic hyperplasia \((BPH)\), 27 localized prostate cancers, and 8 metastatic prostate cancer samples. The quantification of cDNA in each sample was performed by interpolating a \(C_i\) value from a standard curve of \(C_i\) values obtained from serially diluted, commercially prepared cDNA pooled normal prostate samples \((Clontech,\, Palo\, Alto,\, CA)\). This calculated quantity of GOLM1 was then normalized against the quantity of the housekeeping gene glyceraldehyde-3 phosphate dehydrogenase \((GAPDH)\) expressed in that sample. “No–reverse transcription” controls were included to exclude amplification of the genomic DNA. The primer sequences used for GOLM1 were: 5′-CAGCGTGAAAAGCGGAATC-3′ and 5′-TCGGCCTGTGTGAAAAATA-3′. GAPDH primers were prepared as described by Vandesompele et al. \([23]\).

Urine samples were obtained from 333 patients with informed consent after a digital rectal examination before either needle biopsy \((n = 269)\) or radical prostatectomy \((n = 64)\) at the University of Michigan Health System with institutional review board approval as described earlier \((Table\, W1)\) \([19]\). Isolation of RNA from urine and Transplex Whole Transcriptome Amplification (WTA) were as described \([24]\). Quantitative polymerase chain reaction was used to detect GOLM1 and the control transcripts \(PSA\) and \(GAPDH\) from WTA amplified cDNA essentially as described \([24]\). The primer sequences for GOLM1 \([19]\), GAPDH \([23]\), and PSA \([25]\) were previously described. Quantitative polymerase chain reaction was performed on WTA cDNA from urine collected from 129 biopsy-negative patients and 204 patients with prostate cancer \((140\, biopsy-negative\, patients\, and\, 64\, prostatectomy\, patients)\). Samples that had \(PSA\, C_i\) values \(> 28\) were excluded to ensure sufficient prostate cell collection, leading to 124 biopsy-negative and 195 samples from patients with prostate cancer in the analysis. This resulted in a final data
set of samples from 195 patients with prostate cancer (133 positive needle biopsy and 62 radical prostatectomy) and 124 biopsy-negative patients. Threshold levels were set during the exponential phase of the qPCR reaction using Sequence Detection Software version 1.2.2 (Applied Biosystems, Foster City, CA), with the same baseline and threshold set for each plate, to generate threshold cycle (\(C_t\)) values for all genes for each sample. We adjusted GOLM1 was against its mean urine PSA and GAPDH values \((2^{\Delta\Delta Ct} = \frac{\Delta Ct\text{GOLM1}}{\Delta Ct\text{GAPDH}} \times 2^{\Delta\Delta Ct}}\times 1000\)).

**Statistical Analysis**

The receiver-operating characteristic (ROC) curve and a Box plot for GOLM1 were generated using Statistical Package for the Social Sciences 11.5 (SPSS, Inc., Chicago, IL). The area under the ROC curve (AUC-ROC) was then calculated for GOLM1 and serum PSA, respectively.

**Laser Capture Microdissection**

Normal and cancerous prostate epithelial cells were collected using laser capture microdissection (LCM) as described previously [26]. Briefly, for LCM, the SL Microtest device with \(\mu\)CUT software was applied (MMI GmbH, Heidelberg, Germany). A total area of 6 mm\(^2\) was cut for all of the samples and was collected with the aid of the adhesive surface lid from a specially manufactured tube (MMI GmbH). Approximately 10,000 cells collected from each sample were lysed with 30 \(\mu\)l of 5× SDS-reducing sample buffer and clarified by centrifugation at 12,000 g for 10 minutes. The samples were then loaded onto a gel for immunoblot analysis.

**Immunoblot Analysis**

Normal and prostate cancer tissues were homogenized in NP-40 lysis buffer containing 50 mM Tris-HCl, pH 7.4, 1% Nonidet P-40 (Sigma, St. Louis, MO) and complete protease inhibitor cocktail (Roche, Indianapolis, IN). Fifteen micrograms of protein extract was mixed with SDS sample buffer and run on a 10% SDS–polyacrylamide gel under reducing conditions. The separated proteins were transferred onto polyvinylidene fluoride membranes (Amersharm Pharmacia Biotech, Piscataway, NJ), which were incubated for 1 hour in blocking buffer [Tris-buffered saline with 0.1% Tween (TBS-T) and 5% nonfat dry milk]. GOLM1 rabbit polyclonal antibody (raised against amino acids 41-400) was applied at a 1:50,000 dilution in blocking buffer overnight at 4°C. After washing with TBS-T buffer, the membrane was incubated with horseradish peroxidase–linked donkey, anti–rabbit IgG antibody (GE Healthcare Life Sciences, Piscataway, NJ) at 1:5000 for 1 hour at room temperature. The signals were visualized with the ECL detection system (GE Healthcare) and autoradiography. For GAPDH and actin Western blots, the GOLM1 antibody probed membranes were stripped with western reprobe buffer (Geno-tech, St. Louis, MO), blocked in TBS-T containing 5% nonfat dry milk, and incubated with either rabbit anti-GAPDH antibodies (1:50,000 dilution; Abcam, Cambridge, MA) or rabbit anti–actin antibodies (1:5000 dilution; Sigma) for 2 hours. For laser capture microdissected prostate epithelial cell immunoblot, the cells were lysed in sample buffer and analyzed for GOLM1 protein expression by immunoblot analysis as described above.

At the time of initial diagnosis, urine samples \((n = 52)\) from biopsy-proven, clinically localized prostate cancer patients (mean ± SD age, 58.1 ± 0.60 years) were collected with informed consent. Clinical and pathology data from patients with biopsy-proven clinically localized prostate cancer used in this study is provided in Table W2. No treatment was administered to the subjects in the interval between biopsy and urine sample collection. As controls, urine samples from 50 male subjects (mean ± SD, 57.9 ± 0.1 years) with no known history of prostate cancer were collected from the University of Michigan Clinical Pathology Laboratories. Within 2 days of collection, the urine samples were concentrated at 4°C using a Biomax Ultrafree concentrator (5000 MW cutoff; Millipore Corporation, Bedford, MA). The protein content of each sample was estimated with Bradford reagent following the manufacturer’s instructions (Bio-Rad Laboratories, Hercules, CA). The concentrated urine samples were frozen at −20°C until use. Twenty micrograms of total protein from each urine sample was electrophoresed and analyzed for GOLM1 protein expression by immunoblot analysis as described above. The intensity of the 74-kDa GOLM1 band in each sample was scored visually by a researcher who was blinded to the diagnosis of samples and the pattern of loading in the gels. A GOLM1-positive control sample (LNCaP total cell extract) was used for immunoblot experiments. While scoring, the band in the sample lanes with highest reactivity was assigned a score of 4 (highest level of reactivity), whereas absence of a band was scored 0 (non reactivity). Intermediate band intensities were assigned as follows: weak (score = 1), intermediate (score = 2), and high (score = 3). The mean values for GOLM1 reactivity were presented as population mean values with 95% confidence intervals. Student’s t test (2-sided) was used to test for statistically significant differences in GOLM1 reactivity between patients with prostate cancer and control subjects. No adjustment for multiple testing was made during the analysis. P values less than or equal to 0.05 were considered statistically significant. Receiver-operating characteristic curves were used to assess the sensitivity and specificity of urine-associated GOLM1 to detect prostate cancer.

To test the secretion of the GOLM1 protein, a flag-tagged GOLM1 construct was overexpressed in HEK293 Phoenix cells. Cell culture supernatants were collected 12 and 24 hours after transfection. For the analysis, 5 \(\mu\)l of culture supernatant from GOLM1-transfected, vector-transfected, and control protein-transfected cells were run on SDS-PAGE. Immunoblots were probed with either flag or GOLM1 antibodies. Likewise, the presence of GOLM1 was also assessed in the culture supernatants of LNCaP (prostate cell line) and DU145 (prostate cancer cell line). Prostate cancer cell lines were cultured in RPMI medium containing 10% FBS, and the medium was changed to 5% FBS 12 hours before the collection of the supernatant to reduce the bovine serum protein content. Culture supernatants were collected at 24 hours and analyzed by SDS-PAGE and immunoblot analysis with GOLM1-specific antibody. To investigate the specificity of the secretion, the GOLM1 antibody was pre-treated with 5 \(\mu\)g of recombinant GOLM1 protein [15]. Cell-free medium served as the negative control.

**In Vitro Overexpression of GOLM1**

A mammalian expression construct of GOLM1 was generated by subcloning the PCR product into the pACRSPVLPa-(-)loxP-SSP vector (UMICH vector core). Primers used were GOLM1-\(Kpn\)-6-His-F: 3'-GGGAGTTACATCCACCCACACACACACACGGCCTGGAAACCGGCGTCGACG-5' and GOLM1-\(Xba\)-FLAG-R: 5'-GGCTTCTAGATCAAGGCTTCGATCATCGTCTTTGTAGTCAGTTGATTGATTGCGTCTTTTCC-3'. HEK293 Phoenix cells were transfected as
well as vector control using FuGENE6 (Roche) transfection reagent. Culture supernatants were collected 12 and 24 hours after transfection for immunoblot analysis.

**Immunohistochemistry**

For the development of the tissue microarray (TMA), prostate cancer and normal prostate tissues were embedded in paraffin. Study pathologists (M.A.R. and R.M.) reviewed the slides of all cases and designated areas of interest. These slides were used as a template for TMA construction. All TMAs were assembled using the manual tissue arrayer (Beecher Instruments, Silver Spring, MD). At least three tissue cores were sampled from each donor block. Histologic diagnosis of the tissue cores was verified by standard hematoxylin and eosin staining of the initial TMA slide. This radical prostatectomy series is part of the University of Michigan Prostate Cancer Specialized Program of Research Excellence Tissue Core, with informed consent of the patients and prior institutional review board approval. Standard biotin-avidin complex immunohistochemistry was performed using a polyclonal anti-GOLM1 antibody. Digital images were acquired.

**Figure 1.** GOLM1 expression in prostate cancer. (A) GOLM1 transcript levels were collected from DNA microarray analysis of 101 prostate samples. Cy3/Cy5 ratios indicate the expression of GOLM1 in prostate tissue RNA compared to RNA from normal prostate pool. BPH indicates benign prostatic hyperplasia; MET, metastatic prostate cancer; NAP, normal adjacent prostate; PCA, prostate cancer. (B) GOLM1 expression in prostate cancer profiling studies from different publicly available cancer microarray data sets in Oncomine [3,18,26,40] and Yang et al. (unpublished data). Expression array analysis of multiple prostate cancer microarray data sets were collected and analyzed, and statistical significance was calculated. (For details visit: www.oncomine.org). Class 1 represents the GOLM1 expression in normal tissues and class 2 represents GOLM1 expression in cancer. (C) Quantitative polymerase chain reaction confirmation of increased expression of GOLM1 transcripts in prostate cancer. Quantitative polymerase chain reaction was done using RNA from benign, prostate cancer, and metastatic prostate cancer tissue samples. Quantitative polymerase chain reaction of glyceraldehyde-3-phosphate dehydrogenase served as the internal control.
using the BLISS Imaging System (Bacus Laboratory, Lombard, IL) and evaluated using a previously validated Web-based tool (TMA Profiler, University of Michigan, Ann Arbor, MI) [27]. Staining was scored as negative (score = 1), weak (score = 2), moderate (score = 3), and strong (score = 4) based on the intensity of staining of tumor cells using a similar system that has been validated previously [28].

Immunofluorescence Staining and Confocal Microscopy

Prostate tissue sections were soaked in xylene for 1 hour to remove paraffin. Antigen retrieval was achieved by heating the slides in citrate buffer (pH 6.0) for 15 minutes in a pressure cooker. The slides were then blocked in PBS-T containing 5% normal donkey serum for 1 hour. Slides were incubated overnight at 4°C with a mixture of mouse anti–E-cadherin (BD Biosciences, San Diego, CA) and rabbit GOLM1 antibodies at 1:200 and 1:10,000 dilutions, respectively, washed, and followed by secondary antibodies (anti–mouse Alexa 555 and anti–rabbit Alexa 488 at 1:1000 dilutions) for 1 hour. Slides were mounted using Vectashield mounting medium containing DAPI (Vector Laboratories, Burlingame, CA) after washing with PBS-T and PBS. Confocal images were taken with a Zeiss LSM510 META (Carl Zeiss, Göttingen, Germany) imaging system using an ultraviolet, argon, and helium neon 1 light source. The triple color images were exported as TIFF images.

Results

Gene expression profiling studies from our group using prostate tissue RNA demonstrated that GOLM1, a type II Golgi membrane protein–coding gene, was up-regulated and consistently overexpressed in prostate cancer (Figure 1A). Oncomine [29] (www.oncomine.org) analysis of various prostate cancer microarray data sets revealed up-regulation of GOLM1 in prostate cancer samples compared to normal (Figure 1B). Quantitative real-time PCR revealed consistent up-regulation of GOLM1 transcripts in prostate cancer samples confirming the gene expression profiling study findings (Figure 1C). The up-regulation of GOLM1 expression was corroborated at the protein level by immunoblot analysis with lysates from prostate tissues (Figure 2A). There is a marked up-regulation of GOLM1 protein in prostate cancer tissues compared to benign adjacent specimens, whereas metastatic tissue displayed a moderate up-regulation. Further verification and data on cell type–specific expression was obtained by performing immunoblot analysis on laser capture microdissected samples. Epithelial cells from BPH, prostatic intraepithelial neoplasia (PIN), localized prostate cancer (PCA), and hormone refractory metastatic prostate cancer (MET) obtained by LCM were analyzed by Western blot analysis with anti-GOLM1 and subsequently with anti–β-actin (loading control) antibodies (Figure 2B). Laser capture microdissected samples showed up-regulation of GOLM1 protein in PCA and MET samples, demonstrating that the up-regulation observed in whole tissue lysates (Figure 2A) was caused by an increase in GOLM1 expression within the cancer epithelia. The apparent decrease in GOLM1 expression in MET compared to PCA was intriguing and was previously noted by Chandran et al. [30].

Immunofluorescence staining and confocal imaging of prostate tissue section stained with GOLM1-specific antibody not surprisingly revealed a Golgi staining pattern. There was a clear increase in GOLM1 staining in prostate cancer epithelia compared to normal epithelial cells (Figure 3A, pink arrow). High-density TMA analyses indicated moderate-to-strong GOLM1 protein expression in clinically localized prostate cancer samples with predominant cytoplasmic localization (Figure 3B). Expression levels of GOLM1 protein in malignant epithelia were greater compared to those of benign tissue. Scoring of the TMAs indicated that the maximum staining (highest score of 4) was observed in prostate cancer, whereas PIN demonstrated moderate increases when compared to normal prostate epithelium (Figure 3C).

We next sought to detect GOLM1 protein in prostate cell culture supernatant to probe whether it is secreted by prostate cancer cells. A previous study that detected the presence of GOLM1 in serum of hepatocellular cancer patients [17] supported this notion.
Figure 3. Immunofluorescence and immunohistochemical analyses of GOLM1 expression. (A) GOLM1 staining and confocal imaging of prostate cancer epithelial cells. Representative GOLM1 staining is shown in green. E-cadherin (red) was used for costaining, and nuclei were stained with DAPI (blue). Pink arrows indicate the overexpressed GOLM1 protein stained in green. The white arrow indicates a lower level of GOLM1 expression in normal epithelium. (B) The TMA analyses of prostate tissues using anti–GOLM1 antibody. Intense staining of GOLM1 is seen in cancerous prostate epithelial cells. (C) Correlations of GOLM1 staining intensity in benign, PIN, and prostate cancer.
the presence of a specific band in immunoblots of culture supernatant from HEK293 Phoenix cells that overexpressed Flag-tagged GOLM1, with either FLAG antibody or GOLM1-specific antibody (Figure 4A), suggested the presence of a secretory form of GOLM1. This signal was abrogated when GOLM1 antibody was preincubated with purified recombinant protein, showing the specificity of this signal (Figure 4B). In addition, immunoblots also revealed secretion of endogenous GOLM1 by DU145 and LNCaP prostate cell lines, which was inhibited by treatment with 0.5 μM brefeldin A (Figure 4C), a fungal lactone and inhibitor of protein trafficking. This result suggests secretion of Golm1 through the anterograde secretory pathway [20,21]. Brefeldin also blocked the secretion of prostate-specific antigen (PSA) by LNCaP cells (data not shown).

To determine the subcellular localization of GOLM1 in prostate cells, we performed fluorescence immunostaining with GOLM1 antibody using LNCaP cell line. The immunostaining indicated specific Golgi staining as anticipated (Figure 4D, panels i and ii), and colocalization with Golgi marker Golgin 97 confirmed this observation (Figure 4D, panel iii). However, cells treated with brefeldin A showed diffuse staining (Figure 4D, panels iv and v) and colocalization with the endoplasmic reticulum marker protein disulfide isomerase (Figure 4D, panel vi). This observation suggested interference with anterograde transport and increased retrograde transport [20,21] and redistribution of GOLM1 from the Golgi apparatus to the endoplasmic reticulum, which explains the reduced GOLM1 in the culture supernatant after brefeldin A treatment.

Having established the presence of an endogenous secretory form of GOLM1, we further investigated the possibility of detecting GOLM1 in patient serum and urine samples. Immunoblot analysis of urine samples from prostate cancer patients showed a specific band corresponding to GOLM1 comparable in molecular weight to LNCaP lysate. A representative immunoblot is depicted in Figure 5A. Of note, inhibition of GOLM1 reactivity in the urine was observed after preincubation of GOLM1 antibody with the

Figure 4. Detection of GOLM1 protein in cell culture medium. (A) Culture supernatants were collected from HEK293 Phoenix cells overexpressing Flag-GOLM1 construct and immunoblotted with either anti-Flag mouse monoclonal antibody or GOLM1 rabbit polyclonal antibody to detect the presence of GOLM1. Total protein was stained with Ponceau S showing equal loading of culture supernatant. Vector-transfected cells were used as controls. Un-T is culture supernatant from untransfected cells. (B) Immunoblot using anti-GOLM1 antibody that was preincubated with recombinant GOLM1 protein. For immunoblot, culture supernatants from LNCaP and DU145 cell lines were used. (C) Culture supernatants from Du145 and LNCaP prostate cancer cell lines were collected and analyzed by immunoblot. Addition of secretory blocker brefeldin A reduced the GOLM1 protein in the culture supernatant. Representative immunoblots from multiple experiments are shown here. (D) Immunostaining of GOLM1. Panels i and ii show untreated LNCaP cells stained with GOLM1 (green) and E-cadherin (red) antibodies. Nuclei were stained with DAPI (blue). Panel iii shows colocalization of GOLM1 with a Golgi marker protein, Golgin 97 (red). When treated with brefeldin A, GOLM1 shows diffused staining as shown in panels iv and v. Panel vi shows staining of protein disulfide isomerase (red), an endoplasmic reticulum enzyme distribution in brefeldin A–treated cells.
recombinant GOLM1 protein, similar to the results obtained for culture supernatants (Figure 4B; data not shown). The mean score for urine-associated GOLM1 reactivity in prostate cancer patients (mean = 2.77) was significantly greater ($P < .0001$) than in the control subjects (mean = 0.96). Significantly greater percentage of prostate cancer urine samples (75%) had GOLM1 reactivity score in the range of 2 to 4 in contrast to controls (28%; Figure 5B). An ROC curve was generated for GOLM1 reactivity, and an optimum cutoff point was selected at the region where the slope of the curve had the highest value (Figure 5C). At this cutoff point, GOLM1 had the best discriminatory power in distinguishing between urine from prostate cancer patients and control populations (AUC =
Discussion

Gene expression analysis facilitated the identification of gene signatures that are dysregulated in a given cancer. Multiple microarray studies identified GOLM1 (GP73 or GOLPH2), a resident Golgi protein, to be transcriptionally up-regulated in prostate cancer. Here, we demonstrate an up-regulation of GOLM1 in prostate cancer epithelial cells. Further, a secretory form of this protein was identified in culture supernatants of prostate cell lines. On the basis of the detection of the N-terminal FLAG tag in luminal GOLM1, it seems that the release of GOLM1 into the lumen does not involve N-terminal cleavage at the proprotein convertase cleavage site (R52VRRS55) [16]. This suggests that the release mechanism used by prostate cancer cells may differ from hepatocytes. The secretory mechanism of this apparent "full-length GOLM1" is intriguing and remains to be determined. The urinary GOLM1 protein may also be derived from sloughed prostate cancer cells, although such cells are typically only encountered in advanced stages of the disease and after prostate massage [31]. This, however, does not explain the release of intact protein from cultured cells. GOLM1 release from prostate cancer cells could be inhibited by brefeldin A, a protein transport inhibitor [20,21], which suggests that this process could involve secretion. Alternatively, GOLM1 could be released within exosomes [32] or perhaps microvesicles [33]. Regardless of the mechanism, the value of urine GOLM1 measurements as a supplement to serum PSA screening and digital rectal examination is promising and needs to be evaluated.

Larger-scale prospective studies will be necessary to further explore this possibility. Recently, our group has shown that increased GOLM1 transcript in urine, along with SPINK1 and PCA3 transcript expression, and TMPRSS2:ERG fusion status were able to predict prostate cancer outcome. Multivariate regression analysis showed that a multiplex of these biomarkers outperformed serum PSA or PCA3 alone in detecting prostate cancer [19].

Although its function remains uncharacterized, the differential expression of GOLM1 seems to be a common feature of malignancy and has great potential to be developed as a cancer biomarker. For example, Lu et al. [34] identified GOLM1 as one of 187 genes that were consistently dysregulated in 20 common types of cancers, including bladder, breast, colon, endometrium, kidney, liver, lung, melanoma, lymphoma, pancreatic, prostate, and thyroid cancers. In cultured cells, adenovirus infection induces GOLM1 expression through the CtbP domain of the adenoviral E1A protein [35]. Interestingly, recent cell culture studies have demonstrated estrogen-induced changes in GOLM1 expression in the mouse uterus [36] and in androgen-resistant prostate cancer cell lines [37], suggesting a possible hormone regulation in prostate neoplasia. A recent study indicated that there is cancer cell–specific alterations of GOLM1 and MYO6 in prostate cancer [38].

In the absence of any obvious protein domains that would indicate the normal biologic function of GOLM1, gene knock-out studies may be necessary to determine its role in cellular processes. Preliminary in vivo studies in mouse model with a C-terminal truncation suggest a decreased survival and severe renal abnormalities in homozygotes, but no obvious defects in prostate morphology [39]. Cell-specific knockout studies of GOLM1 are currently in progress (Fimmel, in preparation) and may shed light on the function of the protein and its role in prostate and other cancers.

The membrane localization/secretory form of GOLM1 makes it an ideal target for immunotherapy approaches. Further, a better understanding of its function and processing may provide new insights into the transport, sorting, and processing of resident Golgi and secretory proteins. Of particular interest will be the possible role, if any, played by dysregulated GOLM1 expression in PSA secretion through the ER-Golgi-membrane transport pathway.

Acknowledgments

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References


Table W1. Clinical and Pathology Characteristics for the Patients Presenting for Prostate Biopsy or Radical Prostatectomy for the Urines Used for Detection of GOLM1 Transcripts.

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<td>Age, mean ± SE (n = 332)</td>
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<tr>
<td>Gland weight, mean ± SE (n = 70)</td>
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<td>Tumor size, mean ± SE (n = 69)</td>
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<td>PSA, mean ± SE (n = 271)</td>
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Table W2. Clinical and Pathologic Characteristics for Prostate Cancer Patients Used for Urine Analysis of GOLM1 Protein.

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<td>Max dimension of largest tumor,* mean ± SEM (cm)</td>
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*Values available for 50 samples only.  
†Values available for 45 samples only.  
‡Values available for 48 samples only.  
§Values available for 50 samples only.