0022-5347/04/1711-0187/0
THE JOURNAL OF UROLOGY®

Vol. 171, 187–191, January 2004
Printed in U.S.A.
DOI: 10.1097/01.ju.0000101622.74236.94

HEPSIN IS HIGHLY OVER EXPRESSED IN AND A NEW CANDIDATE FOR A PROGNOSTIC INDICATOR IN PROSTATE CANCER

CARSTEN STEPHAN, GEORGE M. YOUSEF, ANDREAS SCORILAS, KLAUS JUNG, MONIKA JUNG, GLEN KRISTIANSEN, STEFFEN HAUPTMANN, TADAAKI KISHI, TERUKAZU NAKAMURA, STEFAN A. LOENING AND ELEFTHERIOS P. DIAMANDIS*

From the Departments of Urology and Pathology, University Hospital Charité, Humboldt University, Berlin, Germany, Department of Pathology and Laboratory Medicine, Mount Sinai Hospital and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada, and Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Athens and National Center for Scientific Research "Demokritos," Athens, Greece

ABSTRACT

Purpose: Other cDNA microarray studies have shown that hepsin is one of the highly over expressed genes in prostate cancer tissue compared with nonmalignant and benign prostatic hyperplasia tissue. We quantitatively analyzed hepsin gene expression with real-time polymerase chain reaction and calculated its relationships with clinicopathological parameters in a large cohort of samples.

Materials and Methods: Matched prostate tissue samples from the cancerous and noncancerous parts of the same prostates were obtained from 90 patients with prostate cancer who underwent radical prostatectomy. Quantitative reverse transcriptase-polymerase chain reaction was performed using LightCycler Fast Start DNA Master SYBR Green I on a LightCycler (Roche Diagnostics GmbH, Mannheim, Germany) system. The ratio of hepsin-to-β-actin (a housekeeping gene) was used to normalize data.

Results: Hepsin over expression in cancerous compared with noncancerous tissue was found in 81 of the 90 patient samples (90%, $p < 0.001$). In 48 patients (53%) hepsin over expression was more than 10-fold in cancerous tissue. The ratio of cancerous-to-noncancerous hepsin expression was significantly higher in the 39 patients with grade 3 tumors compared with the 51 with grade 2 tumors (median 15.5 vs 9.6, $p = 0.031$). For the prognosis a cutoff at the 75th percentile provided a significant difference between patients at lower risk (pT2, G2 and Gleason score less than 7) and higher risk (pT3/4, G3 and Gleason score 7 or greater) for relapse.

Conclusions: This report of the quantitative analysis of hepsin expression, which is the first to our knowledge, shows strong and significant over expression in prostate cancer tissue. Hepsin expression may be a new prognostic marker that could be used for assessing prostate cancer aggressiveness.

KEY WORDS: prostate; prostatic neoplasms; hepsin; serine endopeptidases; tumor markers, biological

Prostate cancer is the most common malignancy of men in the United States. Detecting prostate cancer at earlier curable stages has been facilitated by the measurement of the serine protease prostate specific antigen (PSA) in serum.[1] PSA is the best available serum tumor marker. However, this marker lacks specificity because of increasing levels in benign prostatic diseases. Molecular forms of PSA[2,3] and another member of the human tissue kallikrein family, human glandular kallikrein 2, can increase specificity.[4] However, no current biomarker can predict possible disease progression (PSA failure).[5] Also, pathological stage, tumor volume and margin status, and the amount of Gleason grade 4/5 cancer are important prognostic factors for biochemical failure after radical prostatectomy.[6] Therefore, there is an urgent need for new serum biomarkers that can predict Gleason grade 4/5 prostate cancer.

In recent cDNA microarray studies some interesting genes that are highly over expressed in prostate cancer tissue compared with nonmalignant tissue have been identified.[5,7–9] The transmembrane serine protease hepsin gene was over expressed in all 5 studies. One of these studies that focused especially on aggressive Gleason grade 4/5 tumors revealed 34-fold higher expression of hepsin in cancerous tissue.[6] This finding implicates a possible important role of hepsin in prostate cancer development and progression.

We investigated hepsin expression in matched malignant and benign prostate tissue by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) measurements. We measured hepsin expression in a large cohort to examine the possible relationships to stage, grade and other clinical parameters.

MATERIALS AND METHODS

Study population. Matched prostate tissue samples were obtained from 90 patients with a median age of 63 years (range 48 to 75) who underwent radical prostatectomy for prostatic adenocarcinoma between March 1997 and April 2001 at University Hospital Charité. Nine of the 90 patients who received hormonal therapy 2 to 4 weeks before surgery were also analyzed separately, whereas the remaining 81 did not receive any hormonal therapy. Disease was stages pT2, pT3 and pT4 in 43, 46 and 1 patients, respectively. Further subclassification (pT2a, pT2b, pT3a and pT3b) was not per-

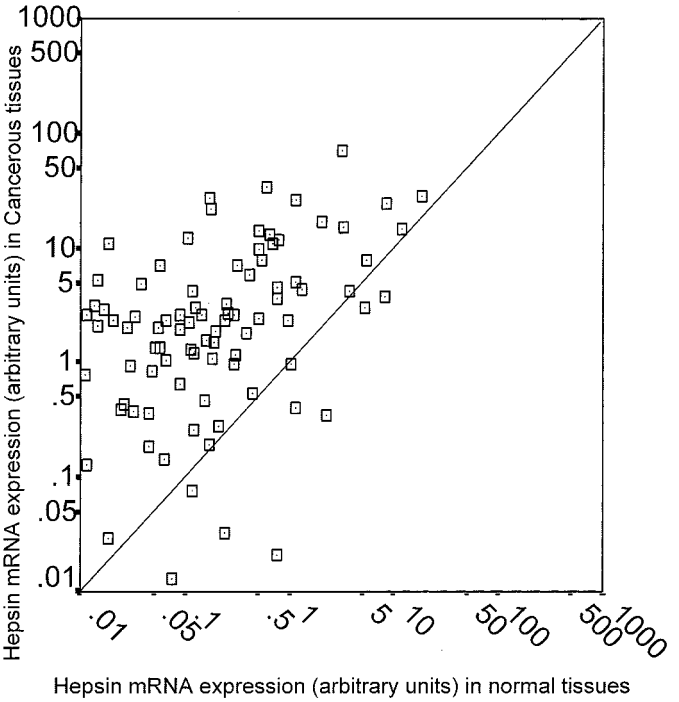
Accepted for publication August 8, 2003.
Study received institutional review board approval.
Supported by MSD, Funds of the German Chemical Industry No. 400770 and Sonnenfeld-Stiftung.
* Correspondence: Mount Sinai Hospital, Department of Pathology and Laboratory Medicine, 600 University Ave., Toronto, Ontario M5G 1X5, Canada (telephone: 416-586-8443; FAX: 416-586-8628; e-mail: ediamandis@mtsinai.on.ca).

formed due to the small number of patients. WHO grade was 2 and 3 in 51 and 39 patients, respectively. Gleason score was less than 7 in 38 patients and 7 or greater in 46, whereas it was not available for 6.

Tissue preparation and primer design. Fresh prostate tissue samples were obtained from the cancerous and noncancerous parts of prostatectomy specimens. Small pieces of tissue were gross dissected by a pathologist immediately after prostate removal, snap frozen and stored in liquid nitrogen until analysis. To ensure that the tissue was malignant or benign histological analysis and confirmation in all tissue pieces was performed by the same pathologist (GK), as described previously.[10] Only tumor samples that were fully surrounded by malignant tissue according to the described analysis were used in this study. We also did not include any samples in which benign prostate glands made up more than 10% of the total mass. This way contamination of the tumor samples with benign glands was minimized. Most tumors were located dorsolateral in the prostate peripheral zone. Tissue characterized as normal was usually taken from the inner zone of the contralateral lobe. Histologically many samples showed mild glandular hyperplasia. Exclusion criteria were prominent inflammatory infiltrates, lack of epithelium due to stromal hyperplasia and prostatic intraepithelial neoplasia.

Tissues were pulverized with a hammer under liquid nitrogen. RNA was extracted using a RNeasy (Qiagen, Inc., Valencia, California) kit according to manufacturer instructions. The RNA concentration was determined spectrophotometrically. Total RNA (2 μg) was reverse transcribed into first strand cDNA using a Superscript II (Gibco BRL, Gaithersburg, Maryland) pre-amplification system. The final volume was 20 μl. Quantitative RT-PCR was performed for hepsin and the housekeeping gene β-actin. The primers used to amplify specific gene product for hepsin were hepsin sense 5′-CGGGACCCCAACAGCGAGGAGAAC-3′ and hepsin antisense 5′-TCGGGGTAGCCAGCACAGAACATC-3′.[9] The primers for β-actin were β-actin sense 5′-ACAATGAGCTGCGTGTGGCT-3′ and β-actin antisense 5′-TCTCCTTAATGTCACGCACGA-3′. Regular PCR was also used for PCR product confirmation (data not shown).

Quantitative RT-PCR. Quantitative RT-PCR was performed on a LightCycler system using SYBR Green I dye, which binds preferentially to double strand DNA. Fluorescence signals, which were proportional to the concentration of the PCR product, were measured at the end of each cycle and immediately displayed on a computer screen.[11] This real-time monitoring of the PCR reaction and the preparation of calibration curves was performed as previously described.[12] For each sample the amounts of the target and endogenous control (β-actin) were determined using a calibration curve. The amount of the target molecule (hepsin) was then divided by the amount of β-actin to obtain a normalized value. All determinations were performed in duplicate. Since each hepsin value was divided by the endogenous control value (β-actin), this ratio was used as an aggregate of hepsin expression. Separate standard calibration curves for β-actin and hepsin were constructed using serial dilutions of a cDNA pool from prostate tissue for β-actin or serial dilutions of plasmid for hepsin. The plasmid for hepsin was prepared according to manufacturer instructions with a TOPO TA Cloning (Invitrogen, Carlsbad, California) kit. Standard curve dilutions were included in each run. LightCycler software automatically calculated the standard curve by plotting the starting dilution of each standard sample vs the threshold cycle. Sample concentrations were then calculated accordingly. Standards for hepsin and β-actin RNA were defined to contain an arbitrary starting concentration and 5 serial dilutions 10-fold each with concentrations defined according to the dilution factor were used to construct the standard curve (see figure).



Hepsin mRNA expression in cancerous (y axis) and normal (x axis) prostatic tissues. Note over expression in approximately 90% of cancer tissues.

PCR reactions for hepsin were done in a reaction mixture consisting of 13.6 μl water, 2.4 μl (4 mM) $MgCl_2$, 0.5 μl (200 ng) primers, 2 μl LightCycler Fast Start DNA Master SYBR Green I and 1 μl cDNA. After the reaction mixture was loaded into a glass capillary cycling conditions were initial denaturation at 95C for 10 minutes, followed by 32 cycles of denaturation at 95C for 1 second, annealing at 57C for 5 seconds and extension at 72C for 20 seconds. The temperature transition rate was set at 20C per second. The fluorescence signal of the specific hepsin product was measured by a single acquisition mode at 89C for 5 seconds after each cycle. This temperature was set after melting curve analysis, which was obtained after amplification, by maintaining the temperature at 72C for 30 seconds, followed by a continuous increase in temperature to 96C at a rate of 0.1C per second with the signal acquisition mode set at step. To verify melting curve results representative samples of the PCR products were run on 1.5% agarose gels, purified and sequenced using vector specific primers with an automated DNA sequencer.

For β-actin PCR reactions were performed in a reaction mixture consisting of 15.2 μl water, 0.8 μl (2 mM) $MgCl_2$, 0.5 μl (150 ng) primers, 2 μl LightCycler Fast Start DNA Master SYBR Green I and 1 μ. cDNA (data not shown). Cycling conditions were initial denaturation at 95C for 10 minutes, followed by 35 cycles of denaturation at 95C for 0 seconds, annealing at 62C for 5 seconds and extension at 72C for 40 seconds. The fluorescence product was measured by a single acquisition mode at 85C for 5 seconds after each cycle. The melting curve was obtained after amplification by maintaining the temperature at 72C for 30 seconds, followed by a continuous increase in temperature to 97C at a rate of 0.1C per second.

Statistical assessment. Statistical analyses were performed with SAS (SAS Institute, Cary, North Carolina) software and SPSS 10.0 for Windows (SPSS, Chicago, Illinois) software. Associations between clinicopathological parameters, such as stage, WHO grade, Gleason score, PSA, percent free PSA and hepsin expression, were analyzed by ANOVA, the Mann-Whitney U or Fisher exact test, the Spearman rank correla-

tion coefficient or the Wilcoxon signed ranks test when appropriate.

RESULTS

The figure shows that hepsin expression was higher in the cancerous tissue part in 81 patients (90%) and lower in only 9 (10%, $p < 0.001$). Table 1 lists a more detailed analysis of these data. Hepsin expression was a mean of 46.1-fold and a median of 11.6-fold higher in the cancerous parts of the respective prostates (table 1). In the 81 patients with higher hepsin expression in the cancerous part over expression was up to 10-fold greater in 33, between 10 and 100-fold greater in 35 and more than 100-fold greater in 13. Absolute hepsin values without correcting with β-actin similarly showed higher expression in the cancerous part in 75 patients and lower expression in 15 ($p < 0.001$).

Comparison of the 9 patients with antiandrogen therapy, of whom all had hepsin over expression, before radical prostatectomy with the remaining 81 without any therapy before surgery, of whom 72 did and 9 did not have hepsin over expression, revealed a significant difference in preoperative PSA only (median 1.20 vs 8.05 ng/ml, $p = 0.001$). All other parameters, including β-actin ($p = 0.76$), hepsin ($p = 0.70$) and the ratio of hepsin-to-β-actin ($p = 0.61$), showed no differences between the 2 groups ($p = 0.09$ to 0.95). Therefore, we considered all patients as 1 group.

The 90 patients had a median age of 63 years, a median preoperative PSA of 7.9 ng/ml, a median percent free PSA of 7.7% and a median prostate volume of 29 ml. Serum PSA (Spearman rank correlation coefficient $r_s = 0.094$, $p = 0.38$), percent free PSA ($r_s = -0.006$, $p = 0.96$), prostate volume ($r_s = -0.127$, $p = 0.25$), age ($r_s = 0.029$, $p = 0.78$) and Gleason score ($r_s = 0.114$, $p = 0.30$) did not correlate with hepsin expression. Hepsin expression was higher in patients with stages pT3 and pT4 vs pT2, showing a trend toward significance ($p = 0.13$, table 2). However, there was a significant difference in hepsin expression between grade 2 vs grade 3 tumors ($p = 0.031$). Hepsin expression was also higher in Gleason score 7 or greater vs less than 7 tumors but it did not attain significance ($p = 0.20$). By searching for an appropriate cutoff to separate patients at high and low risk we found a cutoff equal to the 75th percentile (hepsin expression 28.2). At this cutoff point all parameters (stage, grade and Gleason score) were significantly different between the respective high and low risk groups (table 3).

Together these data demonstrated remarkable over expression of the hepsin gene in cancerous prostatic tissue compared with normal tissue of the same gland in 90% of cases. Over expression was associated with higher grade and stage tumors, and it was independent of preoperative serum PSA.

DISCUSSION

Hepsin, a type II membrane associated serine protease, was originally found as a cDNA clone in a human liver cDNA library.[13] The hepsin gene encodes for a 417 amino acid single chain protein with a molecular mass of 51 kDa.[14, 15] In vitro experimentations implicated hepsin in the maintenance of cellular morphology and hepatic cell growth,[16] and in blood coagulation through human factor VII activation.[17] The primary amino acid sequence of hepsin is highly homologous to trypsin and trypsin-like blood clotting factors, and it is likely to be synthesized as an inactive zymogen. To our knowledge the mechanism of regulation of hepsin activity is not known, although autocleavage has been proposed.[18] Hepsin is not tissue restricted. It is abundantly expressed in the liver, while lower levels are seen in the pancreas, testis, prostate, lung, thyroid and pituitary gland.[15] In immunohistochemical investigations using purified polyclonal antihepsin antibodies[17] specific staining for hepsin exclusively in tumor cell membranes was observed in 7 cases of renal cell carcinoma, whereas in normal renal and other tissues staining was negative.[19] Increased hepsin expression has also been associated with ovarian cancer. The over expression of mRNA was found in 27 of 32 ovarian carcinoma cases (84%) and in 7 of 12 low malignant potential tumors, whereas hepsin was almost never expressed in normal ovarian tissue.[20]

This strong over expression of hepsin in cancer cells is in accordance with the results of this study, in which cancerous tissue over expression was seen in 90% of all prostate cancer cases. Our study used quantitative RT-PCR, which is superior to the qualitative techniques used in a previous study.[20] Recently we successfully applied this method in ovarian and prostate cancer tissues to study the expression of various genes, including *KLK9*[12] and PART-1.

The search for potential new prostate cancer tumor markers has been accelerated by using gene expression profiling with cDNA microarrays. In 2001, 5 studies on this topic were published. For example, 1 study analyzed 4,712 genes and showed that hepsin was the only gene over expressed in all 11 malignant vs 4 nonmalignant samples with a mean 42.5-fold difference.[8] Interestingly hepsin was also found to be expressed at higher levels in prostate intraepithelial neoplasia lesions vs benign prostatic hyperplasia (BPH) tissue, indicating a correlation of hepsin over expression with neoplastic transformation in the prostate. The structure and its homology with other serine proteases strongly imply a possible role for hepsin not only for promoting tumor growth, but also for cancer therapy.[8] Welsh et al evaluated 23 primary cancer tissues and 9 nonmalignant tissues, and selected 400 of 8,920 genes with high and specific expression in prostate tumor tissue.[9] In addition to MIC-1, a member of the transforming growth factor-β superfamily, hepsin was also highly over expressed. Whereas MIC-1 was over expressed in 21 of 24 specimens, hepsin was up-regulated in all primary tumors. In another survey more than 5,520 known genes and 4,464 uncharacterized expressed sequence tags were analyzed in 56 specimens, including (13 BPH, 9 normal prostate tissue and 36 localized and advanced prostate cancer) and 3 cell lines.[7] Hepsin was up-regulated 4.3-fold on microarray and 11.3-fold on Northern analysis.

Immunohistochemical studies revealed strongest expression in high grade prostate intraepithelial neoplasia lesions, followed by primary prostate cancer, hormone refractory prostate cancer and benign prostate tissue to the lowest degree. Lower hepsin expression was seen in patients with

TABLE 1. *Hepsin expression in cancer and noncancer tissues*

	Ca	NonCa	Ca/NonCa Ratio
Mean arbitrary units ± SD	5.76 ± 1.05	1.04 ± 0.28	46.1 ± 10.8
Range (arbitrary units)	0.013–71.4	0.006–18.8	0.03–612
Percentile:			
10	0.18	0.017	0.93
25	0.80	0.05	3.24
50 (median)	2.32	0.17	11.6
75	5.27	0.65	28.2
90	14.8	3.11	130

Values normalized by dividing by respective β-actin value.

TABLE 2. *Hepsin expression in cancerous tissues classified by disease stage, Gleason score and tumor grade*

	No. Pts	Median Hepsin/β-Actin Ratio (arbitrary units)	p Value (Mann-Whitney U test)
Stage:			
PT2	43	9.6	
PT3/4	47	15.3	0.13
Gleason score:			
Less than 7	38	9.6	
7 or Greater	46	14.8	0.20
Unknown	6		
Grade:			
G2	51	9.6	
G3	39	15.5	0.031

TABLE 3. *Relationships between hepsin expression status and histopathological parameters*

Variable	No. Pts	No. Hepsin Neg (%)	No. Hepsin Pos (%)	p Value (Fisher's exact test)
Stage:				
PT2	43	37 (86.0)	6 (14.0)	0.030
PT3/4	47	31 (66.0)	16 (34.0)	
Gleason score:				
7 Less than	38	33 (86.8)	5 (13.2)	0.043
7 or Greater	46	31 (67.4)	15 (32.6)	
Unknown	6			
Grade:				
G2	51	43 (84.3)	8 (15.7)	0.046
G3	39	25 (64.1)	14 (35.9)	

Cutoff point 28.2 arbitrary units equal to 75th percentile with less than 28.2 considered hepsin negative and 28.2 or greater considered hepsin positive.

postoperative PSA failure ($p = 0.03$), higher Gleason scores and larger tumors, indicating an inverse correlation of immunohistochemical hepsin expression with patient prognosis.[7] These observations are in contrast to our results of hepsin mRNA expression.

We generally found that higher hepsin expression was associated with higher Gleason scores, higher WHO grades and higher stages (tables 2 and 3), indicating a positive correlation between hepsin expression and the probability of cancer progression. Using a cutoff at the 75th percentile to obtain the best possible separation all differences were statistically significant (table 3). Of 6,800 genes examined Stamey et al found a subset of 22 up-regulated and 64 down-regulated genes in all 8 BPH and all 9 prostate cancer tissues samples.[6] Since the amount of Gleason grade 4/5 tumor is the strongest predictor of postoperative progression or PSA failure, that investigation focused exclusively on Gleason grade 4/5 prostate tumors. Also, in that study hepsin was the most abundantly over expressed gene with 34-fold higher cancer expression compared with BPH tissue. Stamey et al also ranked differentially expressed genes by functional categories and chromosome localization.

All 5 microarray studies focused on a small number of prostate cancer specimens with different Gleason scores and other patient characteristics. In our study we included 90 patients with variable WHO and Gleason grades as well as stages. To our knowledge our study has the largest tumor sample investigated in this respect to date and the relatively large series allowed us to calculate relationships between aggressive and nonaggressive tumors as well as between pT2 and pT3 tumors. Our results confirm other previous results and demonstrate mean 46.1-fold and median 11.6-fold higher hepsin expression in prostate cancer vs noncancer tissues of the same gland. It also suggested that there is higher hepsin expression in more aggressive and more advanced tumors.

The shortcomings of the current serum markers for prostate cancer include their inability to predict prostate cancer and the rate of postoperative PSA failure. There is a need for new prostate cancer markers that can predict Gleason 4/5 grade tumor. It is important to note that cDNA microarray based gene expression studies provide us with a wealth of data, which must be verified. We found the LightCycler technology to be an excellent tool to investigate and confirm differences in gene expression. Recently published cDNA microarray studies and our first report on quantitative analysis of the most highly over expressed gene, hepsin, are in accordance with the principles of the development of new biomarkers. We believe that this molecule has potential as a diagnostic and prognostic marker for prostate cancer. Further studies toward this goal are warranted.

CONCLUSIONS

Up-regulation of the hepsin gene in advanced and more aggressive tumors suggests a possible role for hepsin protein in prostate cancer progression. Furthermore, hepsin may be a future serum marker for prostate cancer diagnosis and a tissue marker for distinguishing between nonaggressive and more aggressive (Gleason grade 4/5) tumors.

Silke Klotzek and Sabine Becker provided technical assistance.

REFERENCES

1. Polascik, T. J., Oesterling, J. E. and Partin, A. W.: Prostate specific antigen: a decade of discovery—what we have learned and where we are going. J Urol, **162:** 293, 1999
2. Lilja, H., Christensson, A., Dahlen, U., Matikainen, M. T., Nilsson, O., Pettersson, K. et al: Prostate-specific antigen in serum occurs predominantly in complex with alpha 1-antichymotrypsin. Clin Chem, **37:** 1618, 1991
3. Stenman, U. H., Leinonen, J., Alfthan, H., Rannikko, S., Tuhkanen, K. and Alfthan, O.: A complex between prostate-specific antigen and alpha 1-antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: assay of the complex improves clinical sensitivity for cancer. Cancer Res, **51:** 222, 1991
4. Nam, R. K., Diamandis, E. P., Toi, A., Trachtenberg, J., Magklara, A., Scorilas, A. et al: Serum human glandular kallikrein-2 protease levels predict the presence of prostate cancer among men with elevated prostate-specific antigen. J Clin Oncol, **18:** 1036, 2000
5. Stamey, T. A.: Preoperative serum prostate-specific antigen (PSA) below 10 microg/l predicts neither the presence of prostate cancer nor the rate of postoperative PSA failure. Clin Chem, **47:** 631, 2001
6. Stamey, T. A., Warrington, J. A., Caldwell, M. C., Chen, Z., Fan, Z., Mahadevappa, M. et al: Molecular genetic profiling of Gleason grade 4/5 prostate cancers compared to benign

prostatic hyperplasia. J Urol, **166:** 2171, 2001
7. Dhanasekaran, S. M., Barrette, T. R., Ghosh, D., Shah, R., Varambally, S., Kurachi, K. et al: Delineation of prognostic biomarkers in prostate cancer. Nature, **412:** 822, 2001
8. Magee, J. A., Araki, T., Patil, S., Ehrig, T., True, L., Humphrey, P. A. et al: Expression profiling reveals hepsin overexpression in prostate cancer. Cancer Res, **61:** 5692, 2001
9. Welsh, J. B., Sapinoso, L. M., Su, A. I., Kern, S. G., Wang-Rodriguez, J., Moskaluk, C. A. et al: Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. Cancer Res, **61:** 5974, 2001
10. Meyer, A., Jung, K., Lein, M., Rudolph, B., Schnorr, D. and Loening, S. A.: Factors influencing the ratio of free to total prostate-specific antigen in serum. Int J Cancer, **74:** 630, 1997
11. Wittwer, C. T., Ririe, K. M., Andrew, R. V., David, D. A., Gundry, R. A. and Balis, U. J.: The LightCycler: a microvolume multisample fluorimeter with rapid temperature control. Biotechniques, **22:** 176, 1997
12. Yousef, G. M., Kyriakopoulou, L. G., Scorilas, A., Fracchioli, S., Ghiringhello, B., Zarghooni, M. et al: Quantitative expression of the human kallikrein gene 9 (KLK9) in ovarian cancer: a new independent and favorable prognostic marker. Cancer Res, **61:** 7811, 2001
13. Leytus, S. P., Loeb, K. R., Hagen, F. S., Kurachi, K. and Davie, E. W.: A novel trypsin-like serine protease (hepsin) with a putative transmembrane domain expressed by human liver and hepatoma cells. Biochemistry**, 27:** 1067, 1988
14. Tsuji, A., Torres-Rosado, A., Arai, T., Chou, S. H. and Kurachi, K.: Characterization of hepsin, a membrane bound protease. Biomed Biochim Acta, **50:** 791, 1991
15. Tsuji, A., Torres-Rosado, A., Arai, T., Le Beau, M. M., Lemons, R. S., Chou, S. H. et al: Hepsin, a cell membrane-associated protease. Characterization, tissue distribution, and gene localization. J Biol Chem, **266:** 16948, 1991
16. Torres-Rosado, A., O'Shea, K. S., Tsuji, A., Chou, S. H. and Kurachi, K.: Hepsin, a putative cell-surface serine protease, is required for mammalian cell growth. Proc Natl Acad Sci USA, **90:** 7181, 1993
17. Kazama, Y., Hamamoto, T., Foster, D. C. and Kisiel, W.: Hepsin, a putative membrane-associated serine protease, activates human factor VII and initiates a pathway of blood coagulation on the cell surface leading to thrombin formation. J Biol Chem**, 270:** 66, 1995
18. Vu, T. K., Liu, R. W., Haaksma, C. J., Tomasek, J. J. and Howard, E. W.: Identification and cloning of the membrane-associated serine protease, hepsin, from mouse preimplantation embryos. J Biol Chem, **272:** 31315, 1997
19. Zacharski, L. R., Ornstein, D. L., Memoli, V. A., Rousseau, S. M. and Kisiel, W.: Expression of the factor VII activating protease, hepsin, in situ in renal cell carcinoma. Thromb Haemost, **79:** 876, 1998
20. Tanimoto, H., Yan, Y., Clarke, J., Korourian, S., Shigemasa, K., Parmley, T. H. et al: Hepsin, a cell surface serine protease identified in hepatoma cells, is overexpressed in ovarian cancer. Cancer Res, **57:** 2884, 1997