Journal of Clinical Laboratory Analysis 23 : 357–361 (2009)

Human Epididymis Protein 4 is a Biomarker for Transitional Cell Carcinoma in the Urinary System

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Objective: To investigate human epididymis protein 4 (HE4) levels in transitional cell carcinoma (TCC) of the urinary system and its relationship with clinicopathological features.

Methods: 102 patients with TCC, 60 with benign urinary diseases, and 60 healthy controls were included in this study. The HE4 levels were used to analyze different clinicopathologic characteristics and changes between pre- and postsurgical operation.

Results: The HE4 level was significantly increased in patients with TCC compared to patients with benign urinary diseases patients ($P<0.01$) and healthy controls ($P<0.01$), and the level of HE4 in patients with superficial TCC (Tis Ta T1) was significantly higher than that of the benign urogenital group ($P<0.05$) and healthy controls ($P<0.05$). There was a significant difference between HE4 levels in patients before and after operation ($P<0.05$). There was no difference between HE4 levels based on tumor recurrence, clinical TNM stage, lymph node metastasis, or pathological stage ($P>0.05$). The HE4 level was also different between patients with a single tumor versus patients with multiple tumors. The area under the curves of HE4 is 0.821. The sensitivity and specificity of HE4 at a cutoff value of 45.7 pM were 67.6 and 88.3%, respectively.

Conclusions: HE4 may be a screening tool for early diagnosis of TCC in the urinary system, and may become a prognostic marker for TCC in the urinary system. J. Clin. Lab. Anal. 23:357–361, 2009. © 2009 Wiley-Liss, Inc.

Key words: transitional cell carcinoma; human epididymis protein 4 (HE4); urologic diseases; biomarkers

INTRODUCTION

Transitional cell carcinoma (TCC) of the urinary system includes cancer in the renal pelvis, ureters, and bladder, but primarily occurs in the bladder. Bladder cancer is a common urological cancer in both males and females (1). The clinical spectrum of TCC in the urinary system can be divided into three categories: noninvasive/superficial tumors, for which treatment is directed at reducing recurrences and preventing progression to a more advanced stage; invasive lesions, for which one goal of therapy is determining whether the organ can be preserved without compromising survival; and metastatic lesions. Urothelial carcinoma remains a major worldwide healthcare issue largely due to the lack of satisfactory screening methods for early detection of the disease. The importance of early diagnosis in reducing the morbidity and mortality from cancer has led to a search for new sensitive and specific tumor markers. Human epididymis protein 4 (HE4) was first indentified in the epithelium of the distal epididymis and originally predicted to be a protease inhibitor involved in sperm maturation (2,3). The gene, also known as WFDC2, encodes a protein with WAP-type four disulphide core (WFDC) domain (4). Given its homology and comparable transcription profile with known leukocyte protease inhibitors in the WFDC family of proteins, HE4 also presumably has a role in natural immunity (4–6). In malignant neoplasms, gene expression profiling studies have consistently indentified upregulation of HE4 in carcinomas of the ovary (7–15), and several studies have analyzed HE4 protein expression in ovarian neoplasms, providing the opportunity for its application in histopathologic diagnosis (13,14,16,17). Moreover, recent studies have shown elevated HE4 protein levels in serum from patients with ovarian tumors, demonstrating a

Grant sponsor: CanAg Diagnostics AB.

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Received 14 April 2009; Accepted 27 May 2009
DOI 10.1002/jcla.20329
Published online in Wiley InterScience (www.interscience.wiley.com).

similar sensitivity to CA125, but increased specificity for malignant tumors as compared to benign disease (18). However, the examination of HE4 in TCC, benign diseases, and normal controls has not occurred. This search has resulted in the application of HE4 as a candidate urothelial carcinoma biomarker especially for noninvasive/superficial TCC, which is rarely found during a physical examination. Overall, survival may be increased by development of an effective screening strategy that could detect disease in its earliest stage prior to clinical presentation. In this report we observed the relationship between HE4 levels and TCC in the urinary system. We further wished to explore the utility of the HE4 biomarker in a monitoring capacity to detect disease persistence or recurrence.

MATERIALS AND METHODS

Study Populations

One hundred and two patients with TCC of the urinary system, who received operations in Friendship Hospital from September 2008 to April 2009, were enrolled in this study. None of them received radiotherapy, chemotherapy, or immunotherapy before their blood samples were collected. Also, 60 patients with benign urogenital diseases and 60 healthy controls were included in this study. Therefore, the total study population comprised 222 people, 142 males and 80 females. Mean age was 63.4 years (range 34–87). The patients were divided into three groups: the TCC group, benign urogenital diseases group, and healthy controls. Prior to sample collection, appropriate permission was given from the research ethical committee. Peripheral blood was collected from each patient and healthy controls. Patient's data are summarized in Table 1.

Laboratory Methods

Blood was collected in serum separator vacutainer tubes (INSEPACK, Ji Shui Chuang Ge, Beijing, China) as quickly as possible and processed according to the manufacturer's instructions. Specimens were allowed to clot and then centrifuged for 10 min at 1,000*g*. Serum was harvested and stored at -80°C until testing. Frozen samples were mixed thoroughly after thawing and recentrifuged before analysis. Serum samples were tested for HE4 (FUJIREBIOTM Diagnostics, Inc. Sweden) by enzyme immunometric assay.

TABLE 1. Patient Characteristics

Item	TCC ($n = 102$)	Benign ($n = 60$)	Health ($n = 60$)
Gender			
Male	56	39	37
Female	36	21	23
Age(yr)	64.3 ± 10.6	63.7 ± 9.8	61.6 ± 9.4

Statistical Analysis

Diagnostic assay data for normally distributed continuous variables are expressed as mean $\pm$ standard deviation (SD) and continuous variables with non-normal distribution are presented as median and 25–75% percentile. Categorical data are shown as percentages. Differences among the three groups were compared with One-Way ANOVA. The levels of HE4 were compared between two groups using an Independent-Sample T Test method. Differences in patients between presurgical and postsurgical intervention were compared with paired-sample T Test. The cutoffs, sensitivity, and specificity for HE4 were experimentally determined by receiver–operator characteristic (ROC) analysis. A level of $P < 0.05$ was accepted as statistically significant for all statistical comparisons. Statistical analyses were performed using the SPSS software package 10.0 (SPSS, Chicago, IL).

RESULTS

There was no statistically significant difference in age and sex between the three groups ($P > 0.05$). The HE4 concentration was shown in Table 2. The level of HE4 in patients with TCC [66.7 (42.1 ± 108.8)] was significantly higher than that of patients with benign urogenital disease [58.3 (51.8–63.6)] ($P < 0.01$) and healthy controls [37.3 (28.9–44.4)] ($P < 0.01$), and the level of HE4 in patients with superficial TCC (Tis Ta T1) [66.3 (42.4–98.77)] was significantly higher than that of the benign urogenital group [58.3 (51.8–63.6)] ($P < 0.05$) and healthy controls [37.3 (28.9–44.4)] ($P < 0.05$). However, there was no significant difference in between those with benign urogenital diseases and healthy controls (Table 2).

For TCC patients after operation, the level of HE4 is lower than before [66.7 (42.1–108.8) vs. 56.5 (43.8–76.8)] ($P < 0.05$) (Table 3).

TABLE 2. HE4 Levels of the Three Groups

Groups	Cases (n)	HE4 (pM)
Transitional cell carcinoma patients	102	66.7 (42.1–108.8)**
TNM stage		
Tis Ta T1	77	66.3 (42.4–98.77)**
T2–T3	18	70.5 (36.3–147.0)
T4	7	82.4 (42.4–146.0)
Benign urogenital diseases patients	60	58.3 (51.8–63.6)
Healthy controls	60	37.3 (28.9–44.4)

**$P < 0.01$. The HE4 level was significantly higher among patients in superficial TCC (Tis T2–T3 T1) group than the other two groups.

TABLE 3. HE4 Levels of Pre- and Postoperative Patients for Transitional Cell Carcinoma

Groups	Cases (*n*)	HE4 (pM)
Preoperation	102	66.7 (42.1–108.8)
Postoperation	102	56.5 (43.8–76.8)*

*$P<0.05$. Compared with postoperation, the HE4 levels for patients' preoperation were higher.

TABLE 4. Relationship Among the Level of HE4 in Different Clinicopathologic Characteristics

Variables	Cases (*n*)	HE4 (pM)
TNM Stage		
Tis Ta T1	77	66.3 (42.4–98.77)
T2-T3	18	70.5 (36.3–147.0)
T4	7	82.4 (42.4–146.0)
Recurrence		
Yes	47	66.1(43.1–86.0)
No	55	72.1 (39.6–123.7)
Single/multiple tumors		
Single	51	54.0(39.6–82.9)*
Multiple	51	78.8 (51.2–146.0)
Pathological stage		
G1	30	64.3(41.1–93.9)
G2	53	68.8 (41.9–126.0)
G3	19	65.0 (44.4–105.2)
Location of tumor		
Bladder	81	66.1 (41.2–96.5)
Renal pelvic and ureteral	14	86.0 (43.7–140.8)
Lymph node metastasis		
Yes	7	105.2(66.1–146.0)
No	95	65.7 (41.3–98.8)

*$P<0.05$. Compared with the single tumor group, the HE4 levels for the multiple tumors group were higher.

We next addressed whether the level of HE4 was different in TCC patients with different clinicopathologic characteristics. As listed in Table 4, there was a significant difference between patients with a single tumor [54.0 (39.6–82.9)] and multiple tumors [78.8 (51.2–146.0)] ($P<0.05$). There was no difference in HE4 levels among the three TNM stages ($P>0.05$), which are superficial TCC (Tis Ta T1) [78.8 (51.2–146.0)], TCC infiltrating muscle or serosa (T2, T3) [70.5 (36.3–147.0)], and TCC with adjacent tissue invasion (T4) [82.4 (42.4–146.0)]. There was no difference among the three pathological stages [64.3 (41.1–93.9) vs. 68.8 (41.9–126.0) vs. 68.8 (41.9–126.0)] ($P>0.05$) either. Furthermore, there was also no difference among HE4 levels and lymph node metastasis [105.2 (66.1–146.0) vs. 65.7 (41.3–98.8)], recurrence [66.1 (43.1–86.0) vs. 72.1 (39.6–123.7)], and tumor location [66.1 (41.2–96.5) vs. 86.0 (43.7–140.8)] ($P>0.05$).

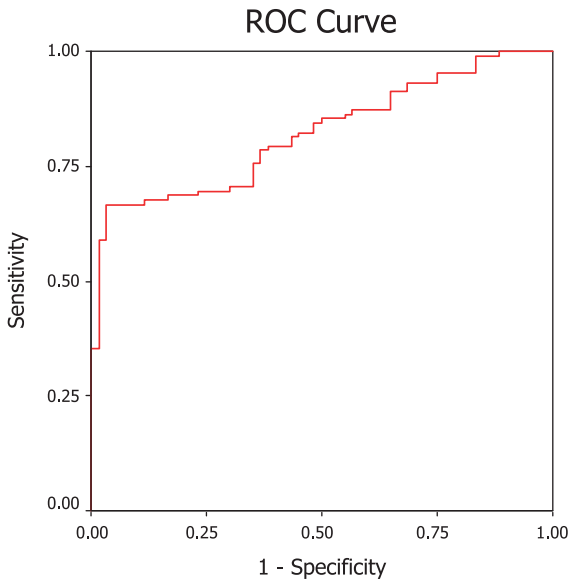

Fig. 1. ROC curves of HE4 for diagnosis of TCC in urinary system.

The optimum diagnostic cutoff point for HE4 levels in this study population was found to be 45.7 pM by ROC analysis (Fig. 1). HE4 levels >45.7 pM demonstrated a sensitivity of 67.6% and a specificity of 88.3% for the diagnosis of HE4. The area under the ROC curve = 0.821 [95% CI: 0.758–0.883]. The standard error of the area under the curve is 0.032.

DISCUSSION

HE4 was first identified in the epithelium of the distal epididymis and originally predicted to be a protease inhibitor involved in sperm maturation (2,3). HE4 (WFDC2) is one of 14 homologous genes on chromosome 20q12–13.1, which encode proteins with a WFDC domain (4). Two of these genes, SLPI and elafin, encode known leukocyte protease inhibitors (19,20), which are co-expressed with HE4 in the upper gastrointestinal, reproductive, and urological tracts (5). The genes at the WFDC locus are variably conserved across species, and presumably play a role in natural immunity with both antimicrobial and anti-inflammatory activity (6). Expression of SLPI and elafin has been identified in various carcinomas, and these genes may play a role in cancer development or progression (21–25). Given its homology and comparable transcription profile with known leukocyte protease inhibitors in the WFDC family of proteins, HE4 also presumably has a role in natural immunity (4–6). Because of its expression in urological tracts (5), recent interest in HE4 has been generated.

For people diagnosed with TCC in the urinary system, the experience of their surgeons and the institutions

where they receive their initial treatment will affect the morbidity and survival rates. For this reason, it is imperative that an accurate screening tool for malignancy is used to triage people at high risk to centers of excellence for their treatment. Equally important, a successful triage tool will allow people at low risk for having a malignancy to stay in their community for their treatment with their primary urologists. The algorithms to screen for malignancy using as presented in this study can be used to classify people into high- and low-risk groups, allowing for the effective triage of people to appropriate surgical centers for their care.

We identified HE4 biomarkers that were elevated in the serum of patients diagnosed with TCC in the urinary system regardless of the TNM stage. HE4 is an 11 kDa precursor to the epididymal secretory protein E4 (26–28). Circulating biomarkers found in the serum of TCC patients may present factors involved in either the cause of or the systemic response to the malignancy. These factors may originate from a number of sources including the tumor itself, the surrounding stroma, or systemic tissues involved in the host response. It is crucial that ongoing work in the field of serum biomarkers is aimed at pinpointing the origins and functional roles of identified biomarkers. From our results, which showed elevated levels of HE4 in the superficial stage, we can conclude that HE4 may become a screening method for early detection of TCC in the urinary system.

This study indicates that the HE4 levels in patients prior to preoperation were significantly higher than postoperation. We consider this result to be preliminary and subject to further evaluation. The efficacy of longitudinal serum monitoring using a biomarker will ideally be evaluated prospectively to determine the role for such a test in the setting of possible disease recurrence.

Compared to patients with a single tumor, the HE4 levels for patients with multiple tumors were higher. Essentially, HE4 helps to stratify patients for treatment by identifying patients with different prognoses, and are important tools in the management of TCC in urinary system.

We found that the ROC plot area of serum HE4 was 0.821 [95% CI: 0.758–0.883]; therefore, HE4 as a biomarker for TCC in the urinary system was significant ($P<0.05$). The optimum diagnostic cutoff point for HE4 levels in this study population was found to be 45.7 pM by ROC analysis. HE4 levels $\geq$45.7 pM demonstrated a sensitivity of 67.6% and a specificity of 88.3% for the diagnosis of TCC. All these results prove that serum HE4 is a good biomarker for the diagnosis of TCC in the urinary system.

In this study, there was a relatively small sample size of patients with TCC in the urinary system ($n = 102$), benign urogenital diseases ($n = 60$), and healthy controls ($n = 60$). Thus, additional studies should be carried out in a larger population to confirm our results. Cut-off values derived from the ROC curves are highly dependent on the study population and might have been different in another set of patients. Thus, the threshold value for HE4 that was used in this study should also be confirmed in larger follow-up studies. In addition, further studies will include larger samples of sera from patients with early TCC, benign disease, and high-risk people participating in TCC screening trials.

These studies are in progress and will enable us and others to define the clinical utility of HE4. Additionally, HE4 is a small secreted glycoprotein. Therefore, it is possible that HE4 is also filtered by the kidneys into the urine. If true, HE4 may also represent a potential target for the development of a urine test for bladder cancer.

ACKNOWLEDGMENTS

This study was supported by CanAg Diagnostics AB (HE4 corporate agent of FUJIREBIO^{TM} Diagnostics, Inc., Sweden), China.

REFERENCES

1. Shirai T. Etiology of bladder cancer. Semin Urol 1993;11:113–126.
2. Kirchhoff C, Habben I, Ivell R, et al. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. Biol Reprod 1991;45:350–357.
3. Kirchhoff C. Molecular characterization of epididymal proteins. Rev Reprod 1998;3:86–95.
4. Clauss A, Lilja H, Lundwall A. A locus on human chromosome 20 contains several genes expressing protease inhibitor domains with homology to wheyacidic protein. Biochem J 2002;368:233–242.
5. Bingle L, Singleton V, Bingle CD. The putative ovarian tumour marker gene HE4 (WFDC2), is expressed in normal tissues and undergoes complex alternative splicing to yield multiple protein isoforms. Oncogene 2002;21:2768–2773.
6. Clauss A, Lilja H, Lundwall A. The evolution of a genetic locus encoding small serine proteinase inhibitors. Biochem Biophys Res Commun 2005;333:383–389.
7. Wang K, Gan L, Jeffery E, et al. Monitoring gene expression profile changes in ovarian carcinomas using cDNA microarray. Gene 1999;229:101–108.
8. Schummer M, Ng WV, Bumgarner RE, et al. Comparative hybridization of an array of 21,500 ovarian cDNAs for the discovery of genes overexpressed in ovarian carcinomas. Gene 1999;238:375–385.
9. Hough CD, Sherman-Baust CA, Pizer ES, et al. Large-scale serial analysis of gene expression reveals genes differentially expressed in ovarian cancer. Cancer Res 2000;60:6281–6287.
10. Ono K, Tanaka T, Tsunoda T, et al. Identification by cDNA microarray of genes involved in ovarian carcinogenesis. Cancer Res 2000;60:5007–5011.
11. Welsh JB, Zarrinkar PP, Sapinoso LM, et al. Analysis of gene expression profiles in normal and neoplastic ovarian tissue samples identifies candidate molecular markers of epithelial ovarian cancer. Proc Natl Acad Sci USA 2001;98:1176–1181.

12. Shridhar V, Lee J, Pandita A, et al. Genetic analysis of early- vs. late-stage ovarian tumors. Cancer Res 2001;61:5895–5904.
13. Schaner ME, Ross DT, Ciaravino G, et al. Gene expression patterns in ovarian carcinomas. Mol Biol Cell 2003;14: 4376–4386.
14. Lu KH, Patterson AP, Wang L, et al. Selection of potential markers for epithelial ovarian cancer with gene expression arrays and recursive descent partition analysis. Clin Cancer Res 2004;10:3291–3300.
15. Gilks CB, Vanderhyden BC, Zhu S, et al. Distinction between serous tumors of low malignant potential and serous carcinomas based on global mRNA expression profiling. Gynecol Oncol 2005;96:684–694.
16. Drapkin R, von Horsten HH, Lin Y, et al. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. Cancer Res 2005;65:2162–2169.
17. Rosen DG, Wang L, Atkinson JN, et al. Potential markers that complement expression of CA125 in epithelial ovarian cancer. Gynecol Oncol 2005;99:267–277.
18. Hellstrom I, Raycraft J, Hayden-Ledbetter M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res 2003;63:3695–3700.
19. Thompson RC, Ohlsson K. Isolation, properties, and complete amino acid sequence of human secretory leukocyte protease inhibitor, a potent inhibitor of leukocyte elastase. Proc Natl Acad Sci USA 1986;83:6692–6696.
20. Wiedow O, Schroder JM, Gregory H, et al. Elafin: An elastase-specific inhibitor of human skin. Purification, characterization, and complete amino acid sequence. J Biol Chem 1990;265: 14791–14795.
21. Devoogdt N, Hassanzadeh Ghassabeh G, Zhang J, et al. Secretory leukocyte protease inhibitor promotes the tumorigenic and metastatic potential of cancer cells. Proc Natl Acad Sci USA 2003;100:5778–5782.
22. Devoogdt N, Revets H, Ghassabeh GH, et al. Secretory leukocyte protease inhibitor in cancer development. Ann NY Acad Sci 2004;1028:380–389.
23. Tian X, Shigemasa K, Hirata E, et al. Expression of human kallikrein 7(hK7/SCCE) and its inhibitor antileukoprotease (ALP/SLPI) in uterine endocervical glands and in cervical adenocarcinomas. Oncol Rep 2004;12:1001–1006.
24. Robinson PA, Markham AF, Schalkwijk J, et al. Increase delafin expression in cystic, dysplastic and neoplastic oral tissues. J Oral Pathol Med 1996;25:135–139.
25. Zhang M, Zou Z, Maass N, et al. Differential expression of elafin in human normal mammary epithelial cells and carcinomas is regulated at the transcriptional level. Cancer Res 1995;55: 2537–2541.
26. Moore RG, Brown AK, Miller MC, et al. Utility of a novel serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus. Gynecol Oncol 2008;110:196–201.
27. Moore RG, Brown AK, Miller MC, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecol Oncol 2008;108:402–408.
28. Scholler N, Crawford M, Sato A, et al. Bead-based ELISA for validation of ovarian cancer early detection markers. Clin Cancer Res 2006;12:2117–2124.