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Serum vascular adhesion protein-1 predicts all-cause mortality and cancer-related mortality in subjects with colorectal cancer

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ABSTRACT

Background: Vascular adhesion protein-1 (VAP-1) participates in inflammation and catalyzes the breakdown of amines to produce aldehyde, hydrogen peroxide, and ammonia. Serum VAP-1 can predict cancer mortality, including colorectal cancer (CRC) mortality, in type 2 diabetic subjects. However, it remains unknown if serum VAP-1 can predict mortality in CRC patients. This prospective cohort study investigates if serum VAP-1 is a novel biomarker for mortality prediction in CRC.

Methods: We enrolled 300 CRC patients. Preoperative serum VAP-1 was measured by time-resolved immunofluorometric assay. They were followed until September 2009 or death, which was ascertained by the National Death Registration System.

Results: The median follow-up period was 4.7 years. Compared with normal counterpart, VAP-1 immunoactivity was upregulated in CRC tissues, especially at the invasion front. Serum VAP-1 can independently predict all-cause mortality (HR: 1.0026, 95% CI: 1.0003–1.0050, $P < 0.05$) and cancer-related mortality (HR: 1.0026, 95% CI: 1.0001–1.0050, $P < 0.05$). A risk score composed of age, gender, carcinoembryonic antigen (CEA) >5 ng/ml, tumor grading, tumor staging, and serum VAP-1 could stratify CRC patients into low-, intermediate-, and high-risk subgroups, with a 5-year mortality rate of 10%, 34%, and 78%, respectively.

Conclusions: Serum VAP-1 predicts mortality independently and improves risk stratification in CRC subjects.

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1. Introduction

Vascular adhesion protein-1 (VAP-1), a 170-kDa homodimeric glycoprotein, is an endothelial adhesion molecule involved in leukocyte rolling, adhesion, and transmigration into sites of inflammation [1]. Another function of VAP-1 is as an enzyme, semicarbazide-sensitive amine oxidase (SSAO), which catalyzes oxidative deamination of primary amines into aldehydes, hydrogen peroxide, and ammonia [2,3]. VAP-1 has a soluble circulating form, which retains its enzymatic function. Serum VAP-1 is released or shed from many tissues such as endothelium, adipocyte, and smooth muscle cells [4,5]. We have previously reported that serum VAP-1 is elevated in subjects with atherosclerosis [6], chronic kidney disease [7], and diabetes [8].

Recently, many studies have investigated the role of VAP-1 in cancers. In gastric cancers, cancer cells show *AOC3* gene amplification [9]. In head and neck, liver, and melanoma tumors, VAP-1 expression is found in intra-tumoral vessels [10–12]. VAP-1 has been shown to enhance tumor growth in mice [13]. In humans, serum VAP-1/SSAO is correlated with angiogenic factors in lung cancers [14] and is more concentrated in metastatic prostatic cancers [15]. In subjects with type 2 diabetes, we have demonstrated that high serum VAP-1 can predict higher cancer-related mortality, including higher colorectal cancer (CRC)-related mortality [16]. However, a recent study reported that low serum VAP-1 was associated with poor prognosis in patients with CRC [17]. Since tumor grading and tumor staging, important prognostic factors, were not adjusted in that study, it remains unclear if serum VAP-1 predicts mortality independently in CRC patients. Therefore, we conducted a prospective cohort study to investigate if serum VAP-1 is an independent prognostic factor in subjects with CRC. We also examined VAP-1 protein expression in cancer tissues and developed a risk score to stratify CRC patients with different risk of death.

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2. Methods

2.1. Patients

From October 2003 to April 2006, we prospectively enrolled patients with pathologically documented colorectal cancer who underwent surgery in the National Taiwan University Hospital. Written informed consents for the collection and analysis of clinical data and specimens were obtained before surgery. Blood and tumor specimens, demographic and clinicopathological data of CRC patients were collected at enrollment. Patients with the following conditions were excluded from this study: 1) patients who refused to give written informed consent for the collection and analysis of blood and surgical specimens; 2) patients with inflammatory bowel disease or with a known family history of familial adenomatous polyposis or hereditary nonpolyposis colorectal cancer; 3) patients who underwent concurrent chemoradiotherapy before surgery; 4) patients who received surgery for recurrent CRC; and 5) patients who died of complications or other diseases during the same hospitalization of surgery. A poorly differentiated (high grade) tumor was defined as one where <10% of the tumor cells formed glands, and mucinous tumor was defined as those containing >50% extracellular mucin. Blood samples for carcinoembryonic antigen (CEA) measurements were taken a few days before operation. A CEA value of >5 ng/ml was considered abnormal. The study protocol was approved by the institutional review boards of the National Taiwan University Hospital. The American Joint Committee on Cancer (AJCC) system was used for TNM staging classification of CRC.

2.2. Treatment for colorectal cancer patients

All patients included in this study were treated by the same surgical team using the same protocol. Surgical resections were done for all patients with stage I, II, and III disease. Patients with resectable stage IV disease were also treated surgically. Adjuvant chemotherapy would be given for patients with stage III and IV disease after surgical resection. Chemotherapy containing 5-fluorouracil, leucovorin and oxaliplatin was the standard regimen for the patients unless contraindicated or found intolerable by the patients. Oxaliplatin was introduced into Taiwan in 1999 and was included in the adjuvant chemotherapy in colorectal cancer in our hospital after October 2003. More than 85% of patients received this regimen as the adjuvant chemotherapy, whereas the rest of the patients received 5-fluorouracil and leucovorin alone. EGFR-targeted therapy (cetuximab) was not included in the standard regimen because it was not covered by the National Health Insurance in Taiwan. Patients who received self-paid EGFR-targeted therapy in either adjuvant or salvage chemotherapy were excluded from analysis in this study.

2.3. Follow-up

All patients had regular follow-ups in our outpatient clinic after surgery and adjuvant chemotherapy to monitor the disease recurrence and metastasis. These patients were observed until death or September 2009. Vital status, date of death, and cause of death of all subjects were ascertained by the National Taiwan Mortality Registry and the medical records in the National Taiwan University Hospital.

2.4. Measurement of serum VAP-1

Serum VAP-1 and its SSAO activity are quite stable. When stored properly at −70 °C, its activity remains intact for at least 2 years [18]. Serum VAP-1 was measured by time-resolved immunofluorometric assay as stated previously. Briefly, the assay utilized a biotin-conjugated monoclonal anti-human VAP-1 antibody (Biotie Therapies Corp.) as a capturer on a streptavidin-coated microtiter plate. Detection of bound serum VAP-1 was performed using a different europium-conjugated anti-human VAP-1 antibody (Biotie Therapies). The time-resolved fluorescence was measured using a fluorometer (Victor^2 Multilabel Counter, PerkinElmer) at 615 nm. Serum VAP-1 concentration was quantified on the basis of a reference sample of highly purified human serum VAP-1 (Biovian Ltd). The R^2 of the standard curves was 0.997–1.000. The intra-assay CVs were 3.7%, 5.2%, and 8.9% for quality control samples with concentrations of 1000, 500, and 100 ng/ml, respectively. The inter-batch CVs from QC samples ranged from 4.4% to 10.2%.

2.5. Immunohistochemistry

The immunohistochemical staining of human CRC for VAP-1 (Santa Cruz Biotechnology) was performed using the Ventana BenchMark autostainer (Ventana). Antigen retrieval was processed by the autostainer with a routine CC1 course (pH 8.0, 1 h; Ventana). Antibodies were incubated on the slides in iVIEW-labeled-conjugate (Ventana) at 25 °C for 90 min. The slides were then incubated in a solution of hydrogen peroxide and 3,3′-diaminobenzidine (DAB) at 25 °C for 8 min, and the sections were counterstained with hematoxylin–eosin. Immunostaining was performed on the specimen of six CRC patients and three patients having colonic diverticulum as negative control to support the validity of staining and identify experimental artifacts. The slides were reviewed by 2 pathologists and the results were expressed as pattern/percentage of positive tumor cells: 0, no staining; 1, focal (1% to 25%); 2, patchy (26% to 50%); 3, diffuse (>50%).

2.6. Statistical analysis

The distributions of continuous variables were examined using the Shapiro–Wilk test. Continuous variables distributed normally were presented as means and standard deviations. Continuous variables with skewed distribution were analyzed after logarithmic transformation and were presented as medians (interquartile ranges). Student's *t* tests and chi-square tests were used to identify the differences in clinical characteristics between survivors and non-survivors. We also used multivariate linear regression analyses to compute adjusted serum VAP-1, also called the marginal means of serum VAP-1. The value was calculated from predictions of a previously fit model with covariates fixed at the mean values.

Survival was estimated using the Kaplan–Meier method and was tested by the log–rank test. Multivariate Cox proportional hazard models were applied to estimate the hazard ratios (HRs) of predictors for all-cause mortality and cancer-related mortality. Variables significantly associated with survival time in univariate Cox proportional hazard models or clinically important variables were put into multivariate analyses. A proportional hazards assumption was evaluated by log–log plots, observed versus expected plots, and was tested for goodness of fit based on Schoenfeld residuals and scaled Schoenfeld residuals. A risk score was constructed by using the regression coefficients in the multivariate Cox proportional hazard model. The receiver operating characteristic (ROC) curve was used to evaluate the performance and to obtain the optimal cutoff values of risk score. To divide the population into high- and low-risk subgroups, the optimal cutoff value was determined through maximizing the Youden index (sensitivity + specificity − 1). To divide the population into high-, intermediate-, and low-risk subgroups, two cutoff values were chosen to maximize the difference of mortality rates between the subgroups without making the different subgroups too small, i.e., <10% of total population.

The contribution of serum VAP-1 to predict 5-year mortality was assessed by the improvement of area under the ROC curves (AUC) and net reclassification index (NRI) [19]. Improvement of AUC was assessed by comparing the AUC of the statistic models with and without serum VAP-1. To calculate NRI, the average 5-year mortality in the 2 risk categories in Fig. 2A was used to divide the subjects into three risk categories (<9%, 9%–45%, and ≥45%). The NRI is estimated as {[(number of events reclassified higher − number of events reclassified lower) / number of events] − [(number of nonevents reclassified higher − number of nonevents reclassified lower) / number of nonevents]}. A two-tailed

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