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Alu-based cell-free DNA: A potential complementary biomarker for diagnosis of colorectal cancer

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ABSTRACT

Objectives: Many patients with colorectal cancer (CRC) present with regional or widespread metastasis, partially reflecting limitations of the current screening programs. This study was aimed to find a complementary marker that can improve the diagnostic accuracy.

Design and methods: Concentrations of cell-free DNA based on *Alu* (*Alu*-based CFD) in 31 unselected CRC patients, 30 intestinal polyp patients and 92 healthy individuals were detected by branch DNA (bDNA). Concentrations of carcinoembryonic antigen (CEA) and carbohydrate antigen 19–9 (CA19-9) were detected by ARCHITECT assay.

Results: There was significant difference in concentrations of CFD between CRC and intestinal polyp patients or healthy individuals ($P<0.0001$). There was no statistically significant difference in CFD in different subgroups of CRC patients with respect to gender, age, tumor site and pathologic stage, suggesting that CFD might be an independent marker relative to CEA and CA19-9. There was a significant correlation between pathologic stage and CEA or CA19-9. Although no significant correlation was observed between pathologic stage and CFD, CFD (the area under the receiver operating characteristic curve (AUC) = 0.904) seemed to be a better indicator to distinguish CRC patients from intestinal polyp patients as compared with CEA (AUC = 0.681) or CA19-9 (AUC = 0.651). CFD was more accurate than CEA or CA19-9 in diagnosing CRC.

Conclusions: Combination of CFD, CEA and CA19-9 may be a better option for the diagnosis of CRC than any of them used alone. Discrimination CRC from intestinal polyp patients with CFD and staging with CEA and CA19-9 may substantially improve the accuracy CRC diagnosis.

Introduction

Colorectal cancer (CRC) is the third most common cancer and one of the leading causes of cancer-related deaths worldwide. Nearly 1 million people worldwide develop CRC each year [1]. With changes in diet and lifestyle, the incidence of CRC has increased yearly. CRC is a heterogeneous complex of diseases caused by destructive genetic/epigenetic alterations that accumulate in a sequential manner through a multistep carcinogenic process [2]. CRC usually experiences no characteristic symptom in the early stage, and is often not diagnosed until the late stage. Surgical resection followed by chemotherapy remains the leading treatment option. However, about 50% CRC patients eventually died of distant metastases [3]. The overall 5-year survival is 57%, and more than 50% of all CRC patients eventually developed metastases. Indeed, the fact that many patients developed regional or widespread metastasis partially reflects the limitations of current screening programs.

Carcinoembryonic antigen (CEA), carbohydrate antigen 19–9 (CA19-9) and CA242 are serum tumor markers commonly used for the diagnosis of CRC, but they are not entirely satisfactory in managing cancer patients because of the lack of sufficient specificity and sensitivity. Misdiagnosis or maldiagnosis is likely when a single marker is used alone [4]. To improve the monitoring and evaluation of CRC patients, there is a need for finding a complementary marker that can assure accurate diagnosis and early detection of recurrence.

Abbreviations: CRC, colorectal cancer; CFD, cell-free DNA; bDNA, branch DNA; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19–9; ROC, receiver operating characteristic; AUC, the area under the ROC curve.

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Abnormally high levels of cell-free DNA (CFD) can be detected in plasma and serum of various cancer patients. Elevated CFD levels were often detected before the appearance of colorectal cancer [5,6]. Persistent high or increased DNA level in circulating blood may therefore signal a relapse and is probably a sign of poor prognosis [7]. CFD has the potential of replacing DNA from invasive and laborious tissue biopsies [8].

The objective of our study was to assess the diagnostic value of serum CFD based on *Alu* in CRC patients by bDNA, and explore the feasibility of combination CFD with CEA or CA19-9 in CRC diagnosis.

Methods

Subjects and blood samples

This study comprised 92 healthy controls (60 male and 32 women, age 18–56 years), 31 CRC patients (18 men and 13 women, age 46–83 years) and 30 intestinal polyp patients (22 men and 8 women, age 38–82 years) from the Departments of General Surgery and Digestive System at Affiliated Hospital of Nantong University (Nantong, China) between August 2010 to May 2011. All CRC patients had confirmed histological diagnosis of cancer with the examiners blind to the clinical conditions of the patients. The locations of the colorectal neoplasms were as follows: 7 cancers were located in the right colon, 5 in the left colon, 4 in the transverse colon, and 15 in the rectal tract. The distribution of the tumors was according to the TNM classification system. The 32 healthy control subjects were selected from volunteer blood donors of Nantong Blood Center who had no history of autoimmune diseases, tissue injuries or traumas and whose hematological–biochemical profiles were normal at the time of examination.

All samples were anonymous and the study protocol was approved by the local ethics committee. Blood samples (4–5 mL) were collected into serum separator tubes containing clot activation additive and a barrier gel (Vacuette, Kresmunster, Austria) immediately before surgical resection. The whole blood was centrifuged at 1600*g* for 10 min, and the serum was stored at −80 °C until use.

Quantitation of CFD

CFD level was quantitated by branch DNA (bDNA) technology established by Jing et al. of our research team [9].

Quantitation of CEA and CA19-9

CEA and CA19-9 concentrations were determined by ABBOTT ARCHITECT I2000 SR. A CEA value of >5 ng/mL and a CA19-9 value of >37 ng/mL were considered abnormal.

Statistics

Statistical analysis was performed with GraphPad Prism v5.0 software. The results for *Alu*, CEA and CA19-9 concentrations are presented as the median with the 25th and the 75th percentile values. Inter-group comparison of nonparametric quantitative data was performed with the Mann–Whitney test. Comparison of nonparametric quantitative data more than 2 groups was performed with the Kruskal–Wallis test. Correlation was analyzed by using the Spearman test. Receiver operating characteristic curves (ROC) were generated to assess the diagnostic accuracy of each parameter, and the sensitivity and specificity of the optimum cut-off point were defined as values that maximized the area under the ROC curve (AUC). 95% was defined as confidence interval of ROC. All statistical tests were two-sided, and a *P* value of smaller than 0.05 was considered statistically significant.

Results

Cell-free DNA in patients with CRC and intestinal polyps and healthy individuals

Thirty-one unselected patients with primary CRC were enrolled at the affiliated hospital of Nantong University. The bDNA assay was used to measure the level of CFD based on *Alu*. The median concentrations of CFD based on *Alu* in patients with CRC and intestinal polyps and healthy individuals were 1067.0 ng/mL (interquartile range 349.7–1713.0 ng/mL), 195.9 ng/mL (interquartile range 96.6–463.8 ng/mL) and 175.2 ng/mL (interquartile range 109.5–305.3 ng/mL), respectively. There was significant difference between the CRC and intestinal polyp patients or healthy individuals ($P<0.05$), but there was no statistical difference between the intestinal polyp patients and healthy individuals ($P>0.05$) (Fig. 1).

Cell-free DNA and clinical characteristics of CRC patients

Alu-based CFD, CEA and CA19-9 levels in different subgroups of CRC patients with respect to gender, age, tumor site and TNM staging are illustrated in Table 1. There was no statistically significant difference in *Alu-based* CFD in these different groups: gender ($P>0.05$), age ($P>0.05$), tumor site ($P>0.05$), and pathologic stage ($P>0.05$) (Fig. 2).

Evaluation of cell-free DNA, CEA and CA19-9 in diagnosis of CRC

To evaluate *Alu-based* CFD as a biomarker, Spearman correlation analysis was performed. No significant correlation was observed between *Alu*-based CFD and pathologic stage ($r=0.107$, $P>0.05$), CEA ($r=0.232$, $P>0.05$) or CA19-9 ($r=-0.158$, $P>0.05$), while there was a significant correlation between pathologic stage and CEA ($r=0.548$, $P<0.05$) or CA19-9 ($r=0.605$, $P<0.05$) (Table 2).

The ROC curve was plotted to identify a cut-off value that could distinguish between CRC patients and healthy controls. The maximal likelihood ratio (59.4) was 634.9 ng/mL, and this value was chosen as the optimal cut-off. At the optimal cut-off value, the level of *Alu*-based CFD presented a 64.5% sensitivity and a 98.9% specificity in separating CRC patients from healthy controls with an AUC of 0.904. Other ROC

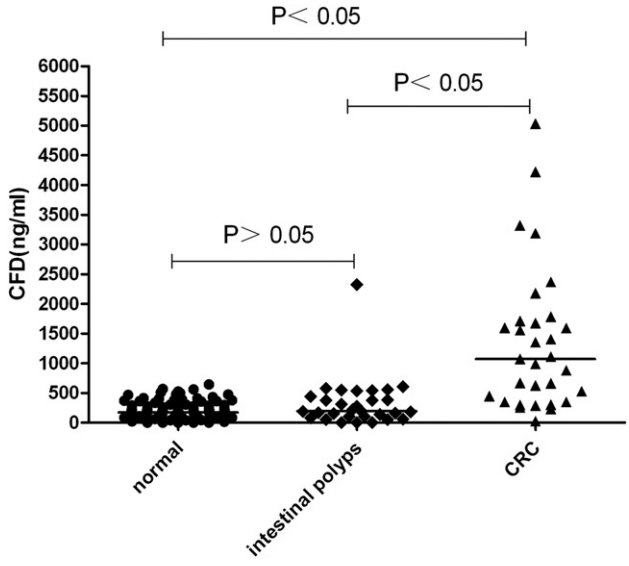

Fig. 1. *Alu*-based CFD levels in healthy controls, intestinal polyp and CRC patients. Mann–Whitney test was used in this figure. CFD levels were measured in 31 unselected CRC patients, 30 unselected intestinal polyp patients and 32 healthy individuals. The results for the CFD levels are presented as the median. Horizontal lines indicate the median for each group. CFD: cell-free DNA; CRC: colorectal cancer.