Cancer Diagnostics

Increased Integrity of Free Circulating DNA in Sera of Patients with Colorectal or Periampullary Cancer: Direct Quantitative PCR for ALU Repeats

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Background: Cell-free DNA circulating in blood is a candidate biomarker for malignant tumors. Unlike uniformly truncated DNA released from apoptotic nondiseased cells, DNA released from dead cancer cells varies in size. We developed a novel method to measure the ratio of longer to shorter DNA fragments (DNA integrity) in serum as a potential biomarker for patients with colorectal cancer (CRC) or periampullary cancers (PACs).
Methods: Sera from 32 patients with CRC (3 stage I, 14 stage II, 6 stage III, and 9 stage IV patients), 19 patients with PACs (2 stage I, 9 stage II, 1 stage III, and 7 stage IV patients), and 51 healthy volunteers were assessed by quantitative real-time PCR of ALU repeats (ALU-qPCR) with 2 sets of primers (115 and 247 bp) amplifying different lengths of DNA. We used serum directly as a template for ALU-qPCR without DNA purification. DNA integrity was determined as ratio of qPCR results of 247-bp ALU over 115-bp ALU.
Results: ALU-qPCR had a detection limit of 0.01 pg of DNA. Eliminating DNA purification reduced technical artifacts and reagent/labor costs. Serum DNA integrity was significantly increased for stage I/II and III/IV CRC and stage I/II and III/IV PACs ($P = 0.002$, $P = 0.006$, $P = 0.022$, and $P < 0.0001$, respectively). ROC curves for detecting CRC and PACs had areas under the curves of 0.78 and 0.80, respectively.
Conclusions: Direct ALU-qPCR is a robust, highly sensitive, and high-throughput method to measure serum DNA integrity. DNA integrity is a potential serum biomarker for detection and evaluation of CRC and PACs.
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Colorectal cancer (CRC)[4] and periampullary cancers (PACs), including primarily pancreatic cancer, were the third and fourth leading causes of cancer-related deaths in the United States between 1995 and 2000 (1). The death rate for advanced CRC remains unsatisfactory, and the mortality from pancreatic cancer is among the worst of all cancers. Approximately 80% of patients with pancreatic cancer present with unresectable disease; thus, the 5-year relative survival rate is only 4% (1). The key for improvement of prognosis and treatment is early diagnosis of malignancy; however, most CRCs and PACs are asymptomatic in the early stages of the disease. Screening with established tumor markers for gastrointestinal cancers, such as carcinoembryonic antigen or CA19-9, has limited efficiency because of erratic detection and increased concentrations in benign disorders (2, 3). Therefore, a widely applicable sensitive screening tool is clinically desired.

Free circulating DNA in serum or plasma is a proposed diagnostic and prognostic biomarker for malignant tumors (4–6). Increased DNA integrity in plasma, derived from threshold cycle numbers assessed by quantitative real-time PCR (qPCR) for 2 amplicons (400 bp and 100 bp) of a specific gene, was reported to indicate the presence of gynecologic and breast cancers (7). The premise is that DNA released from necrotic malignant cells varies in size, whereas DNA released from apoptotic cells is uniformly truncated into 185- to 200-bp fragments (8). Because the main source of free circulating DNA in healthy individu-

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[4] Nonstandard abbreviations: CRC, colorectal cancer; PAC, periampullary cancer; qPCR, quantitative real-time PCR; PBL, peripheral blood leukocyte; IQR, interquartile range; and AUC, area under the curve.

als is apoptotic cells, a preponderance of longer DNA fragments could be a marker for malignant tumor detection *(9)*. However, assessment of the integrity of free circulating DNA is not yet practical for clinical use because the sensitivity and specificity of these methods have not been validated. A potential limitation may be the purification of DNA from serum or plasma, which decreases DNA yield. DNA loss may be inversely dependent on fragment size, which would affect DNA integrity values.

Recently, we developed a robust, highly sensitive, high-throughput method to measure the integrity of free circulating DNA in serum by qPCR for ALU repeats in a 0.1-μL equivalent volume of serum as a template without DNA purification. The ALU is the most abundant repeated sequence in the human genome, with a copy number of ~1.4×10^6 per genome *(10, 11)*. ALU sequences are short interspersed elements, typically 300 nucleotides in length, that account for more than 10% of the human genome *(12)*. ALU elements multiply within the genome in a retroposition process through RNA polymerase III–derived transcripts from evolution *(13, 14)*; therefore, qPCR of ALU repeats with a properly designed primer set can dramatically increase the sensitivity of size-dependent DNA measurement. In this pilot study of CRC and PACs, we describe this method in detail and validate the practical utility of serum DNA integrity as a sensitive tumor biomarker.

Materials and Methods

SERUM SAMPLES AND CLINICOPATHOLOGIC INFORMATION

Serum samples from 32 patients with CRC, 19 patients with PACs, and 51 healthy volunteers were assessed. The PAC group consisted of 15 patients with pancreatic ductal adenocarcinomas, 2 with ampullary cancers, 1 with acinar cancer, and 1 with duodenal cancer. Blood was drawn before therapeutic intervention. Patients were selected by the database coordinator based on those patients treated between 1997 and 2005 at the John Wayne Cancer Institute (JWCI) and at the University of California Los Angeles (UCLA). All patients in this study gave consent according to the guidelines set forth by JWCI and UCLA Institutional Review Board committees. Of 32 patients with CRC, 3 had American Joint Committee on Cancer stage I disease, 14 had stage II, 6 had stage III, and 9 had stage IV. Of 19 patients with PACs, 2 had American Joint Committee on Cancer stage I disease, 9 had stage II, 1 had stage III, and 7 had stage IV. Staging was based on postoperative pathology findings for resected cancers or diagnostic imaging for unresectable cancers. Clinicopathologic data were obtained after Institutional Review Board approval for all patients.

QUANTITATIVE PCR OF ALU REPEATS

The target for ALU-qPCR in this study was a consensus sequence of human ALU interspersed repeats (see Fig. 1

in the Data Supplement that accompanies the online version of this article available at http://www.clinchem.org.content/vol52/issue6). We designed 2 sets of primers for ALU repeats: the primer set for the 115-bp amplicon (ALU115) amplifies both shorter (truncated by apoptosis) and longer DNA fragments, whereas the primer set for the 247-bp amplicon (ALU247) amplifies only longer DNA fragments. The sequences of the ALU115 primers were as follows: forward, 5′-CCTGAGGTCAGGAGTTC-GAG-3′; reverse, 5′-CCCGAGTAGCTGGGATTACA-3′. The ALU247 primers were as follows: forward, 5′-GTG-GCTCACGCCTGTAATC-3′; reverse, 5′-CAGGCTGGA-GTGCAGTGG-3′.

The reaction mixture for each ALU-qPCR consisted of a template, 0.2 μM each of forward primer and reverse primer (ALU115 or ALU247), 1.0 U of *iTaq* DNA polymerase (Bio-Rad Laboratories), 0.02 μL of fluorescein calibration dye (Bio-Rad Laboratories), and a 1× concentration of SYBR Gold (Molecular Probe) in a total reaction volume of 20 μL with 5 mM Mg^{2+}. Real-time PCR amplification was performed with precycling heat activation of DNA polymerase at 95 °C for 10 min, followed 35 cycles of denaturation at 95 °C for 30 s, annealing at 64 °C for 30 s, and extension at 72 °C for 30 s in an iCycler iQ Real-Time PCR Detection System (Bio-Rad Laboratories). The absolute equivalent amount of DNA in each sample was determined by use of a calibration curve with serial dilutions (10 ng to 0.01 pg) of gently prepared genomic DNA obtained from peripheral blood leukocytes of a healthy volunteer. A negative control (without template) was run in each reaction plate. All qPCR assays were performed in a blinded fashion without knowledge of the specimen identity, and the mean values were calculated from triplicate reactions. PCR products were electrophoresed on 2% agarose gels to confirm product size and the specificity of the PCR.

DNA integrity was calculated as the ratio of qPCR results with the 2 primer sets: Q_{247}/Q_{115}, where Q_{115} and Q_{247} are the ALU-qPCR results obtained with the ALU115 and ALU247 primers, respectively. Because the annealing sites of ALU115 are within the ALU247 annealing sites, the qPCR ratio (DNA integrity) would be 1.0 when template DNA is not truncated and 0.0 when all template DNA is truncated into fragments smaller than 247 bp. Because the ALU115 primers can amplify most fractions of circulating DNA, ALU-qPCR results obtained with ALU115 primers represent the absolute amount of DNA.

SERUM PREPARATION AND DIRECT ALU-qPCR

We collected 1-mL blood samples into CORVAC serum separator tubes (Sherwood-Davis & Geck). Samples were processed within 6 h as follows: the blood was separated by centrifugation (1000*g* for 15 min) and passed through a 13-mm serum filter (Fisher Scientific) to remove potential contaminating cells. Serum was immediately stored at −80 °C. To deactivate or eliminate proteins that bind to template DNA or DNA polymerase and might invalidate

qPCR results, we mixed 20 μL of each serum sample with 20 μL of a preparation buffer that contained 25 mL/L Tween 20, 50 mmol/L Tris, and 1 mmol/L EDTA. This mixture was digested with 16 μg of proteinase K solution (Qiagen) at 50 °C for 20 min, followed 5 min of heat deactivation and insolubilization at 95 °C. After subsequent centrifugation at 10 000g for 5 min, 0.2 μL of the supernatant (containing 0.1-μL equivalent volume of serum) was used as a template for each direct ALU-qPCR reaction.

EVALUATION OF ALU-qPCR
Because the ALU-qPCR method used in this study was newly developed, we initially evaluated the performance of ALU-qPCR itself, using purified DNA or serum as a template.

We evaluated the sensitivity and linearity of ALU-qPCR with ALU115 or ALU 247 primers by use of a serially diluted, known amount of purified DNA obtained from peripheral blood leukocytes (PBLs) of a healthy volunteer. In addition, we compared the results of ALU-qPCR with ALU115 primer for serum DNA with the results obtained with the PicoGreen (Molecular Probes) reagent, which is a sensitive fluorescent nucleic acid stain for quantifying double-stranded DNA. Serum DNA from 15 healthy volunteers and 8 patients with PACs (evaluation set) was extracted and purified by a conventional technique; 500 μL of each separated and filtered serum was digested with 400 μg of proteinase K solution (Qiagen) along with 10 g/L sodium dodecyl sulfate, and DNA was purified by phenol–chloroform–isoamyl alcohol and ethanol precipitation as described previously (6).

We assessed the reproducibility of direct ALU-qPCR with ALU115 and ALU247 primers by triplicate reactions using a 0.1-μL equivalent volume of each serum of evaluation set.

We also evaluated the interfering effect of substances in serum on direct ALU-qPCR. Samples containing 10 ng of purified PBL DNA (P), 0.1-μL equivalent volume of serum (S), and a mixture of them (P+S) were prepared for each serum or evaluation set. DNA amounts in (P), (S), and (P+S) were quantified separately by ALU-qPCR with ALU115 and ALU247 primers. The interfering effect of serum on ALU-qPCR was calculated as follows: $1.0 - [Q_{115\,(P+S)} - Q_{115\,(S)}]/Q_{115\,(P)}$ for ALU115 primers and $1.0 - [Q_{247\,(P+S)} - Q_{247\,(S)}]/Q_{247\,(P)}$ for ALU247 primers, where $Q_{115\,(x)}$ and $Q_{247\,(x)}$ are the ALU-qPCR results for sample x with ALU115 and ALU247 primers, respectively. The interfering effect of serum on DNA integrity was calculated as follows: $1.0 - [Q_{247\,(P+S)} - Q_{247\,(S)}]/[Q_{115\,(P+S)} - Q_{115\,(S)}]$.

STATISTICAL ANALYSIS
We used Dunnet's multiple comparison to compare the absolute concentration or integrity of serum DNA with clinicopathologic characteristics and used ROC curve analysis to assess the discriminating ability of assessments. The statistical package SAS JMP, Ver. 5.1 (SAS Institute Inc.) was used for statistical analyses. A P value <0.05 (two-tailed) was considered significant.

Results

SENSITIVITY, LINEARITY, AND REPRODUCIBILITY OF ALU-qPCR
To evaluate the performance of ALU-qPCR for length-dependent quantification of DNA, we tested the sensitivity, linearity, and reproducibility of ALU-qPCR, using purified genomic DNA.

The calculated relative efficiency of ALU-qPCR in relation to fragment length of template DNA is shown in Fig. 1. The solid line shows the estimated efficiency of ALU-qPCR with ALU115 primer set: 0% for DNA fragments <115 bp and >90% for DNA fragments >1150 bp; the dotted line is for the ALU247 primer set: 0% for fragments <247 bp and 90% for fragments >2470 bp. As a result, DNA fragments between 115 and 247 bp, which covers the length of DNA cleaved by the apoptotic process, can be amplified with ALU115 primers but not with ALU247 primers.

The threshold cycles of ALU-qPCR with the ALU115 or ALU247 primers on serially diluted genomic DNA (10 ng to 0.01 pg) obtained from PBLs of a healthy volunteer are shown in Fig. 2A of the online Data Supplement. With both primer sets, linearity was maintained in the 10^6 range, and logarithmic regression lines had $R = 0.998$ for ALU115 and $R = 0.999$ for ALU247 primers; the detection limit was as low as 0.01 pg, equivalent to approximately a 1/300 copy of genome in a single cell. Agarose gel electrophoresis of PCR products obtained with the ALU115 and ALU247 primer sets on 10, 1, 0.1, and 0.01 pg of genomic DNA templates with 28 cycles of thermal cycling confirmed that the target sequence was specifically amplified without major aberrant bands (see Fig. 2B in the online Data Supplement).

We also evaluated the sensitivity of ALU-qPCR, using clinical samples from 15 healthy volunteers and 8 patients
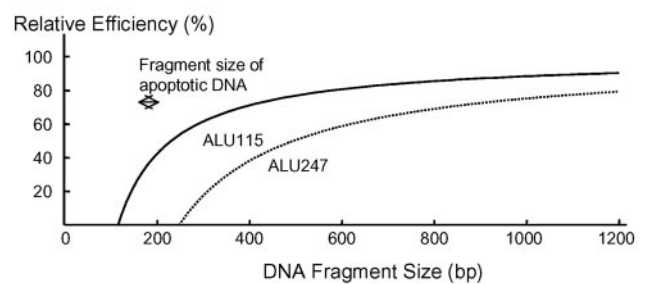

Fig. 1. Calculated relative efficiency of DNA quantification in terms of DNA length.

The estimated efficiency of the ALU115 primers is shown as a *solid line*: 0% for fragments <115 bp and >90% for fragments >1150 bp. The estimated efficiency of the ALU247 primers is shown as a *dotted line*: 0% for fragments <247 bp and >90% for fragments >2470 bp. The size of DNA released from apoptotic cells is indicated by the *double-headed arrow*.

with PACs (Fig. 2). The amount of DNA purified from 500 μL of each serum specimen in the evaluation set was measured 2 ways: by the PicoGreen assay, which consumed one-tenth of the total extracted DNA; and by ALU-qPCR with ALU115 primers, which consumed only one-five thousandth of the total extracted DNA. Most of the serum samples from healthy volunteers contained too little DNA for accurate quantification by the PicoGreen assay. In contrast, ALU-qPCR had sufficient sensitivity and linearity for serum DNA quantification. ALU-qPCR results for specimens having relatively high serum DNA concentrations showed 1:1 linearity with the PicoGreen assay (Fig. 2).

We evaluated the reproducibility of direct ALU-qPCR by use of triplicate reactions using 0.1-μL equivalent volume of each serum specimen in the evaluation set. The median replicate CVs of the DNA concentrations quantified with for ALU115 and ALU247 primers were only 9% [interquartile range (IQR), 7%–14%] and 17% (IQR, 8%–24%), respectively. These CV values were equivalent to those for qPCR with specific primers for other genes (data not shown).

INTERFERING EFFECTS OF SERUM ON DIRECT ALU-qPCR

Because unpurified serum DNA directly used as a template for ALU-qPCR can inhibit the reaction efficiency, we tested its interfering effect on ALU-qPCR.

The median interfering effects of serum on direct ALU-qPCR with ALU115 and ALU247 primers were 0.09 (IQR, 0.03–0.20) and 0.24 (IQR, 0.09–0.37), respectively. The median interfering effect on DNA integrity was 0.12

Fig. 2. DNA quantification by PicoGreen and ALU-qPCR.

DNA was conventionally extracted and purified from 500 μL of sera obtained from 15 healthy volunteers and 8 patients with PACs, and its amount was quantified by ALU-qPCR with ALU115 primers and the PicoGreen method. A one-tenth and a one-five thousandth amount of total purified DNA (50-μL and 0.1-μL equivalent volume of serum) were used for each quantification by the PicoGreen method and ALU-qPCR method, respectively. The *filled arrowhead* on the *y* axis indicates the lower limit of the PicoGreen method. The *diagonal dotted line* indicates the assumed fit line of the 2 methods if they have no lower limits.

(IQR, 0.08–0.18). Because the high sensitivity of ALU-qPCR lowered the requirement of serum template for ALU-qPCR, the interfering effect of serum was limited.

ABSOLUTE CONCENTRATION AND INTEGRITY OF SERUM DNA

Healthy volunteers. The mean (SD) age of 51 healthy volunteers (18 males and 33 females) was 48 (11) years. The mean (SE) absolute serum DNA concentration in healthy volunteers was 0.34 (0.25) ng/μL, and the mean serum DNA integrity was 0.13 (0.01). The absolute concentrations and integrity of serum DNA in healthy volunteers were independent of sex and age.

Patients with CRC. The mean (SD) age of 32 patients with CRC (19 males and 12 females) was 66 (14) years. Mean absolute serum DNA concentrations in patients with stage I/II and stage III/IV CRC were 1.63 (0.43) and 1.73 (0.45) ng/μL, respectively, which were significantly higher than in healthy volunteers ($P = 0.006$ and 0.004, respectively; Fig. 3A). The area under the ROC curve (AUC) for distinguishing patients with CRC from healthy volunteers by absolute DNA concentrations was 0.75 (Fig. 3B). The mean (SD) serum DNA integrity in patients with stage I/II and stage III/IV CRC was 0.22 (0.02) and 0.22 (0.02), respectively, significantly higher than in healthy volunteers ($P = 0.002$ and 0.006, respectively; Fig. 4A). The AUC of the ROC curve for discriminating patients with CRC from healthy volunteers by serum DNA integrity was 0.78 (Fig. 4B). The ROC curves for serum DNA integrity and absolute serum DNA concentration were similar, indicating that serum DNA integrity may be equivalent to absolute serum DNA concentration with respect to CRC detection.

Patients with PACs. The mean (SD) age of 19 patients with PACs (12 males and 7 females) was 68 (9) years. Mean absolute serum DNA concentrations in patients with stage I/II and stage III/IV PACs were 0.84 (0.53) and 0.66 (0.62) ng/μL, respectively. There was no significant difference between cancer patients and healthy volunteers ($P = 0.85$ and 0.98, respectively; Fig. 5A). The AUC of the ROC curve for discriminating patients with PACs from healthy volunteers by absolute DNA concentrations was only 0.59 (Fig. 5B). The mean serum DNA integrity in patients with stage I/II and stage III/IV PACs was 0.22 (0.03) and 0.30 (0.03), respectively, significantly higher than in healthy volunteers ($P = 0.022$ and $P < 0.0001$, respectively; Fig. 6A). The AUC for the ROC curve for discriminating patients with PACs from healthy volunteers by serum DNA integrity was 0.80 (Fig. 6B). This was greater than the AUC for absolute serum DNA concentration, indicating that serum DNA integrity was more informative than absolute serum DNA concentration with respect to the detection of PACs.
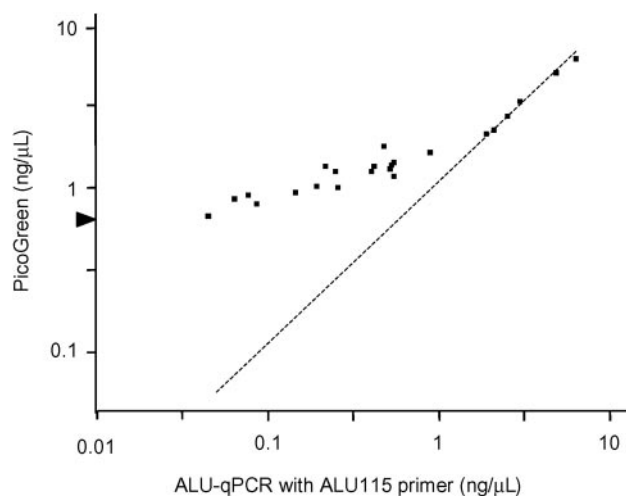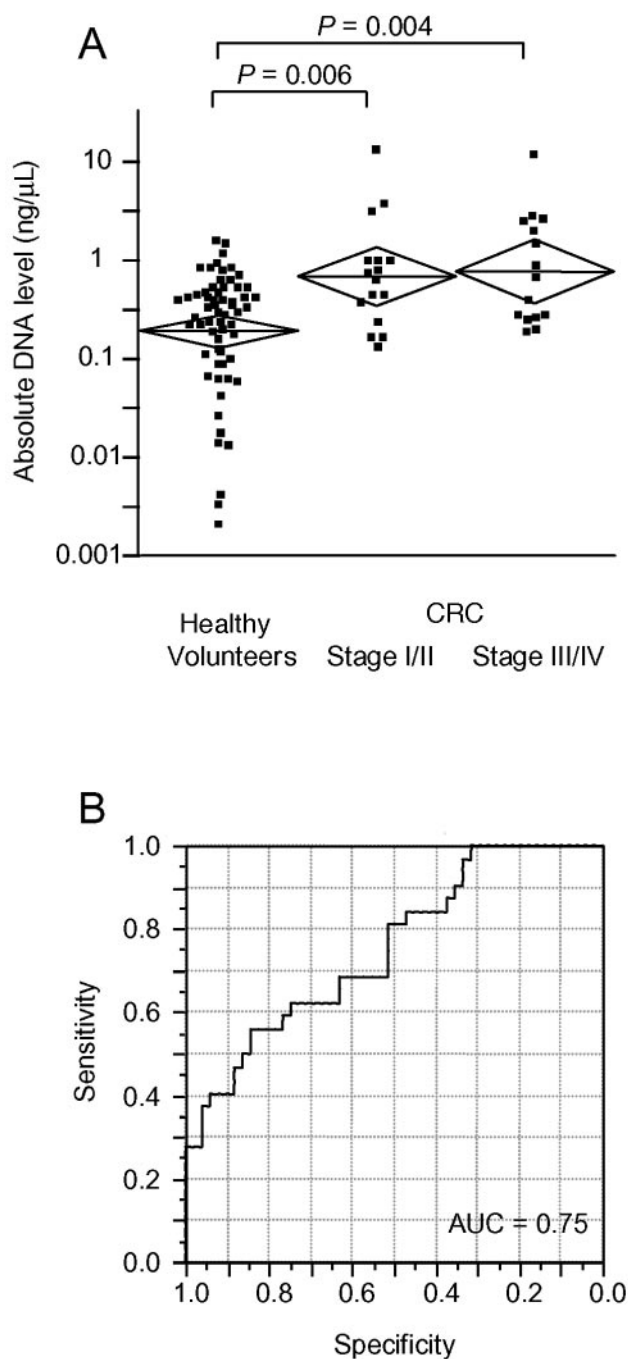
Fig. 3. Concentrations of free circulating DNA in sera from controls and patients with CRC.

(*A*), absolute concentrations of free circulating DNA in serum of healthy volunteers and patients with stage I/II and stage III/IV CRC. *Diamonds* indicate the 95% confidence intervals, and *horizontal lines* indicate the sample mean. Patients with stage I/II and stage III/IV CRC had significantly higher absolute serum DNA concentrations (P = 0.006 and 0.004, respectively). (*B*), ROC curve for discriminating patients with stage I-IV CRC from healthy volunteers (AUC = 0.75). Sensitivity was 0.41 at a specificity of 0.90.

Fig. 4. Integrity of free circulating DNA in serum of controls and patients with CRC.

(*A*), integrity of free circulating DNA in serum of healthy volunteers and patients with stage I/II and stage III/IV CRC. *Diamonds* indicate 95% confidence intervals, and *horizontal lines* indicate sample means. Patients with stage I/II and stage III/IV CRC had significantly higher serum DNA integrity (P = 0.002 and 0.006, respectively). (*B*), ROC curve for discriminating patients with stage I-IV CRC from healthy volunteers (AUC = 0.78). Sensitivity was 0.56 at a specificity of 0.90.

Discussion

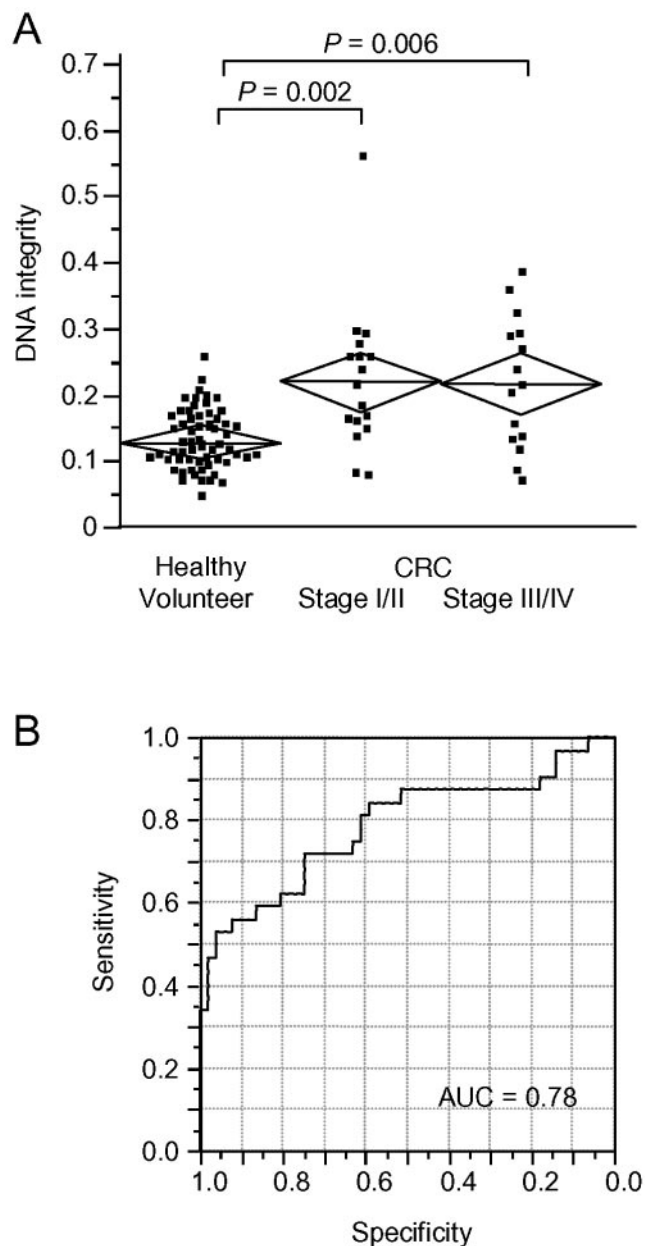Free circulating DNA in serum/plasma is a promising biomarker of cancer because it contains DNA released from dead tumor cells. Detection of cancer-specific so-matic sequence variations for genes such as *K-ras* has been demonstrated in plasma/serum of patients with CRC or pancreatic cancer *(15–17)*. Cancer detection by quantifying the absolute concentration of free circulating DNA in serum/plasma has also been reported *(18–21)*. Plasma DNA integrity was reported to be a predictor of gynecologic and breast cancer existence *(7)*.
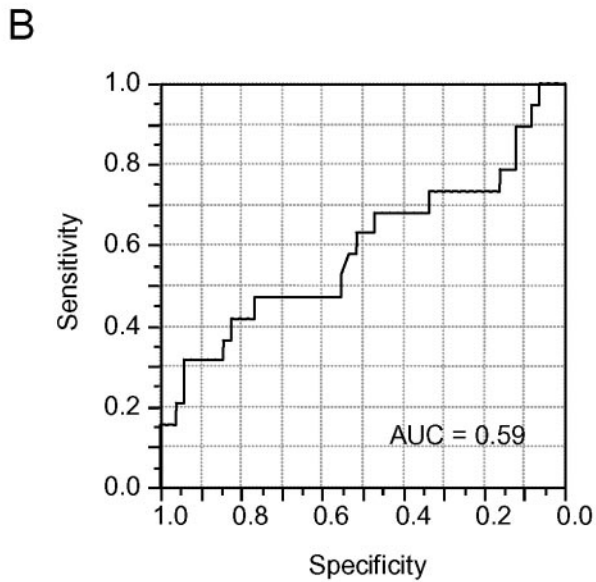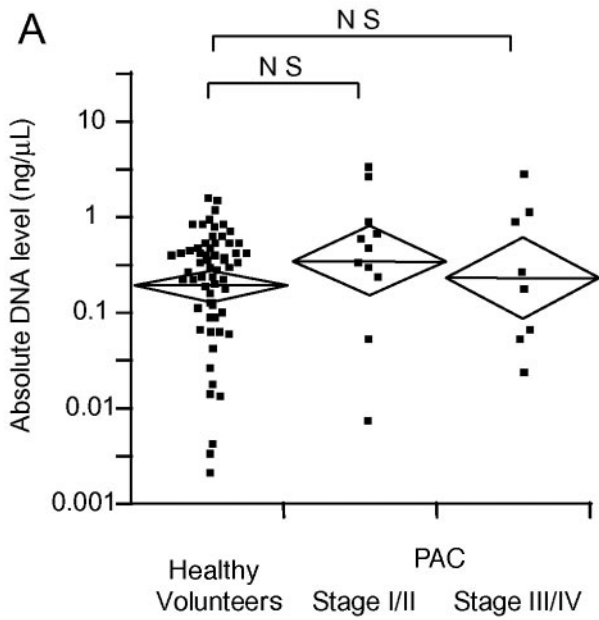
DNA integrity may represent cancer cell death and

Fig. 5. Concentrations of free circulating DNA in serum of controls and patients with PACs.

(*A*), absolute concentrations of free circulating DNA in serum of healthy volunteers and patients with stage I/II and stage III/IV PACs. *Diamonds* indicate 95% confidence intervals, and *horizontal lines* indicate sample means. No significant increases in absolute serum DNA concentrations were observed in patients with stage I/II and stage III/IV PACs. (*B*), ROC curve for discriminating patients with stage I-IV PACs from healthy volunteers (AUC = 0.59). Sensitivity was 0.31 at a specificity of 0.90.

Fig. 6. Integrity of free circulating DNA in serum of controls and patients with PACs.

(*A*), integrity of free circulating DNA in serum of healthy volunteers and patients with stage I/II and stage III/IV PACs. *Diamonds* indicate 95% confidence intervals, and *horizontal lines* indicate sample means. Patients with stage I/II and stage III/IV PACs had significantly higher serum DNA integrity ($P = 0.022$ and $P < 0.0001$, respectively). (*B*), ROC curve for discriminating patients with stage I-IV PACs from healthy volunteers (AUC = 0.80). Sensitivity was 0.58 at a specificity of 0.90.

thus could be a widely applicable biomarker for cancer existence or progression. However, difficulties in handling the very low concentrations of DNA in serum/ plasma have been a technical barrier for practical appli-
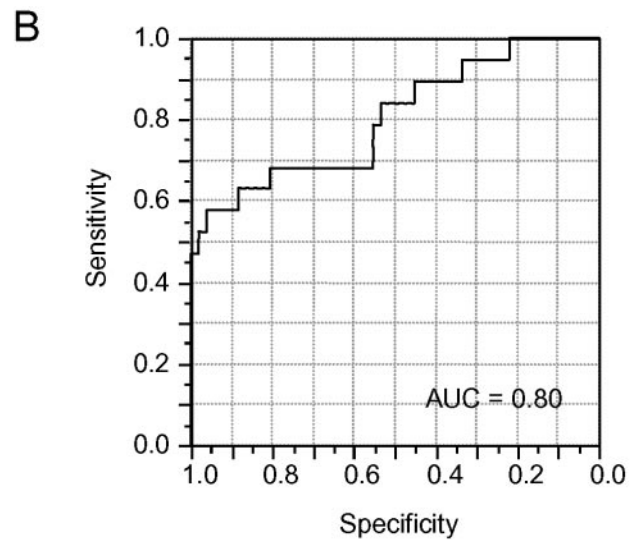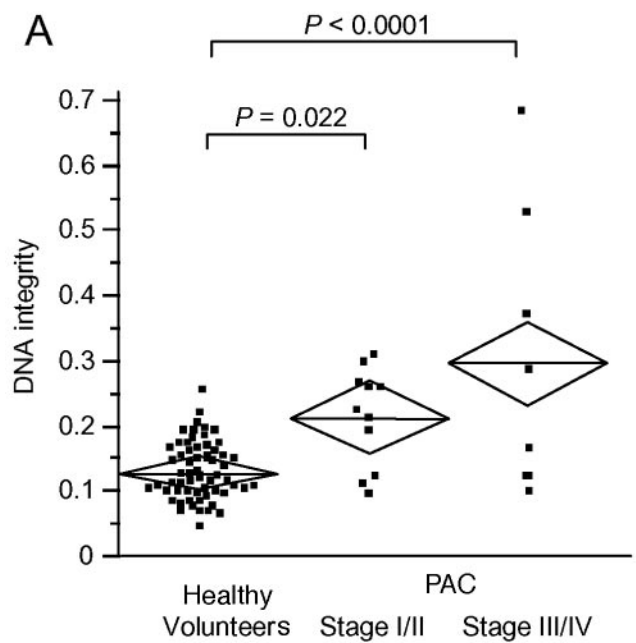
cations. DNA purification steps introduce loss of DNA, which in itself is a problem in the assessment of free circulating DNA. In addition, because the recovery of serum/plasma DNA depends on DNA fragment size, it becomes a critical fluctuating factor for DNA integrity. To

overcome these problems, we developed the ALU-qPCR method to directly measure the absolute amount and integrity of DNA in serum.

Because the ALU is the most abundant repeated sequence (10, 11), ALU-qPCR has sufficient sensitivity for direct assessment of serum. Elimination of the DNA purification step in direct ALU-qPCR stabilized the ratio of shorter and longer DNA fragments in serum. In addition, a calibration curve created by simultaneously performed qPCR on serially diluted genomic DNA in each reaction plate minimized the variance of ALU-qPCR results between reaction plates. Elimination of DNA purification also reduced the reagent and labor costs for the assessment, which is an important factor for implementation of screening tools. For large-scale future assessments, direct ALU-qPCR is easily adaptable to robotic automation. The extremely small volume of serum needed for this assessment is compatible with its use as a screening tool.

The concentrations of free circulating DNA are 4- to 6-fold higher in serum than in plasma (22–24). Because this difference does not reflect contaminated extraneous DNA during separation, serum is a better specimen source for circulating disease-related DNA (24). In addition, the reproducibility of direct ALU-qPCR when we used plasma as a template was inferior to the reproducibility when we used serum. However, during serum separation, cell lysis of PBLs may cause an artificial increase in DNA integrity. Increases in serum DNA were reportedly observed with overnight clotting after blood drawing (22) but not at 8 h (data not shown). We therefore processed blood within 6 h after blood drawing.

This pilot study demonstrates that serum DNA integrity is a clinically useful biomarker for detecting CRC and PACs. Serum DNA integrity was significantly increased even in localized CRC and PACs. It may therefore be useful for mass screening of malignant diseases. However, because any necrotic or mechanically ruptured cells release longer DNA fragments, patients with nonneoplastic diseases such as injury (25), acute inflammation, or infarctions may have high serum/plasma DNA integrity. In addition, pregnancy may cause a false positive because of fetal DNA in the maternal bloodstream (26). Such conditions may represent exclusion criteria for the assay as a screening tool for malignancy. Whether sera from patients with benign lesions such as colonic polyps have higher DNA integrity is unknown. A future study will help determine this.

The absolute concentration of serum DNA had a predictive value for CRC but not for PACs in this pilot study. We therefore consider serum DNA integrity a better molecular biomarker than absolute serum DNA concentration. There are some possible reasons for the wide distribution in absolute DNA concentrations seen in Fig. 3A, such as aggressiveness of the disease, rapid turnover of the tumor, or immune response to the tumor. However, we did not find any specific conditions in the patients studied. Sera from certain cases of advanced cancers in this study showed very high ALU-qPCR values with ALU115 primers, which lowered the serum DNA integrity. In such cases, absolute serum DNA may be a better serum biomarker than DNA integrity. Therefore, a combined index of absolute concentration and integrity of serum DNA may decrease false negatives for cancer detection. For example, the {index of [mean +1.5 × SD of log(absolute DNA concentration)] or (mean + 1.5 × SD of DNA integrity)} achieves 92% specificity with 63% sensitivity for detection of stage I-IV CRC/PACs. To derive a definite index for clinical use, a large-scale clinical trial is needed.

DNA integrity has potential to help in CRC screening and surveillance, which now require colonoscopy, an invasive and relatively expensive examination (27). Although subsequent early operative intervention has improved CRC survival (28), a disproportionately high percentage of patients (15%–20%) with early-stage CRC suffer from local and distant recurrences (1). Surveillance mandates expensive follow-up procedures that are not cost-effective for the vast majority of postoperative patients (29).

PACs, specifically pancreatic cancer, are the fourth most common cause of cancer-related deaths in the United States (1). Of the estimated 32 190 new cases of pancreatic cancer in 2005, 80%–90% presented with clinically apparent metastatic disease or radiographic evidence of unresectability (1). Unfortunately, effective screening programs for pancreatic cancer have not been developed, and patients are often detected incidentally or after development of symptoms. Assessment of DNA integrity as an easy, simple, inexpensive screening method that could provide the impetus to initiate more aggressive radiographic evaluation. For those who are fortunate to have early diagnosis, surgical resection offers the only hope for cure. Despite adherence to rigorous surgical technique and histopathology-based diagnosis, up to 80% of patients will suffer early, locoregional recurrences (30, 31). Therefore, DNA integrity can also be used as a postoperative surveillance tool.

In conclusion, direct ALU-qPCR is a simple, robust, highly sensitive, and high-throughput method for measuring the integrity of free circulating DNA in serum. The elimination of DNA purification steps reduces technical artifacts and reagent and labor costs. Serum DNA integrity was significantly increased in patients with CRC and PACs and thus is a promising biomarker for detecting CRC and PACs. However, large-scale prospective studies are needed to establish the clinical utility of this index. In addition, the high sensitivity of direct ALU-qPCR suggests that it may be applicable for measurement of DNA concentration or DNA integrity in other human body fluids.

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