quantified, but they could potentially be particularly useful in developing countries.

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References

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Preferential Isolation of Fragmented DNA Enhances the Detection of Circulating Mutated k-ras DNA, Mengjiong Wang, Timothy M. Block, Laura Steel, Dean E. Brenner, and Ying-Hsiu Su* (1 Department of Biochemistry and Molecular Pharmacology Jefferson Center for Biomedical Research, Thomas Jefferson University, 700 E. Butler Ave., Doylestown, PA 18901; 2 Departments of Internal Medicine and Pharmacology, 2150B Cancer and Geriatrics Center, University of Michigan Medical Center, Ann Arbor, MI; * author for correspondence: fax 215-489-4920, e-mail Yinghsiu.Su@jefferson.edu)

In the course of studying the incidence of k-ras mutations in DNA in the circulation of patients with colorectal disease, we observed that our ability to detect mutated k-ras DNA in serum varied with the method of DNA isolation. Two methods of DNA isolation were used in this study. One was the QIAamp DNA Blood Midi Kit (Qiagen, Inc.), which has been widely used for isolating circulating DNA from serum or plasma (1–5). The other is the modified Guanidine/Promega Resin (G/R) method that was developed to isolate DNA from urine (6).

To compare the applicability of the Qiagen and G/R methods for isolation of circulating DNA, we purified DNA from aliquots of serum from six patients with known mutant k-ras-positive colorectal disease (colorectal cancer or adenomatous polyps) and analyzed it for the presence of mutated k-ras DNA. As negative controls, serum samples from six patients with no detectable k-ras mutations in their disease tissues were subjected to DNA isolation and subsequently analyzed for k-ras mutations. Briefly, DNA from two aliquots of serum from each patient was isolated by the Qiagen method according to the manufacturer’s instructions or by the G/R method, as follows. Two volumes of 6 mol/L guanidine thiocyanate were mixed with the serum by inverting the mixture eight times. One milliliter of resin (from the Wizard DNA isolation reagent set; Promega) was then added, and the mixture was incubated for 2 h at room temperature with gentle mixing. The resin–DNA complex was transferred to a minicolumn (provided in the reagent set) and washed, and the DNA was eluted with H2O. Isolated DNA was quantified by real-time PCR on a LightCycler (Roche Biochemicals) with human albumin primers (forward, 5′-CCG TGG TCC TGA ACC AGT TA-3′; reverse, 5′-GTC GCC TGT TCA CCA AGG AT-3′) at an annealing temperature of 55 °C. As calibrators for quantification, serially diluted genomic DNA was used. The limit of detection with the albumin primers was 0.015 ng.

DNA derived from 50 μL of serum was used to assay for k-ras DNA, mutated in codon 12, by the modified restriction endonuclease-enriched PCR (RE-PCR) assay (7). The PCR product of this modified RE-PCR was 87 bp with an assay detection limit of 15 copies of the mutant k-ras per 100 ng of wild-type DNA per reaction (data not shown). The k-ras mutation was detected in circulating DNA isolated by the G/R method in five of six patients with known k-ras mutations in their diseased tissue. However, when circulating DNA was isolated by the
Qiagen method, the k-ras mutation was detected in only one of these six patients (see Table 1). As controls, circulating DNA was isolated from patients (patients 7–12 in Table 1) with no detectable mutated k-ras sequences in their diseased tissues, and none scored positive for a k-ras mutation. The failure of the Qiagen method to detect mutated k-ras in 5 of the 6 DNA preparations from patients containing detectable mutated k-ras was not attributable to the low yield of DNA because the Qiagen method gave higher DNA yields in aliquots from 8 of 12 individuals tested (Table 1). Thus, the G/R method significantly enhanced the sensitivity of detection of the mutated k-ras DNA compared with the Qiagen method, as analyzed by the Fisher exact test (P = 0.0389).

A recent report (5) suggested that circulating Epstein–Barr virus DNA molecules from patients with nasopharyngeal carcinoma or lymphoma were relatively short, with 87% of them shorter than 181 bp. We therefore hypothesized that the higher detection rate of circulating mutated k-ras DNA by the G/R method compared with the Qiagen method might be attributable to differences in the sizes of DNA isolated. It is known that mutations in the k-ras gene trigger the apoptosis pathway [as reviewed in Ref. (8)] and that DNA fragmentation occurs. It has been shown that serum cell-free circulating DNA the size of nucleosomal DNA (3). Mutated k-ras DNA detected in the circulation could be in the form of fragmented DNA. It is possible that DNA isolated by the G/R method is enriched in fragmented DNA the size of nucleosomal DNA, possibly derived from cells undergoing apoptosis.

### Table 1. DNA yields and presence of mutated k-ras DNA in serum samples isolated by the Qiagen or G/R method from patients with colorectal disease.

<table>
<thead>
<tr>
<th>Patient/ (diagnosis)</th>
<th>DNA yield, µg/L</th>
<th>Mutated k-ras DNA assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QIAamp</td>
<td>G/R</td>
</tr>
<tr>
<td>1 (CRC)</td>
<td>26.7</td>
<td>95</td>
</tr>
<tr>
<td>2 (Admn polyps)</td>
<td>26.9</td>
<td>&lt;0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 (Admn polyps)</td>
<td>8.01</td>
<td>1.76</td>
</tr>
<tr>
<td>4 (Admn polyps)</td>
<td>19.2</td>
<td>&lt;0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 (Admn polyps)</td>
<td>3.81</td>
<td>7.7</td>
</tr>
<tr>
<td>6 (Admn polyps)</td>
<td>36</td>
<td>95</td>
</tr>
<tr>
<td>7 (CRC)</td>
<td>70.7</td>
<td>1.0</td>
</tr>
<tr>
<td>8 (Admn polyps)</td>
<td>11.3</td>
<td>1.5</td>
</tr>
<tr>
<td>9 (Admn polyps)</td>
<td>62</td>
<td>2.1</td>
</tr>
<tr>
<td>10 (Admn polyps)</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>11 (Admn polyps)</td>
<td>1.8</td>
<td>&lt;0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 (Admn polyps)</td>
<td>5.3</td>
<td>11.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> All human samples were collected according to the protocol approved by the Institutional Review Board. Patients 1–6 have detectable mutated k-ras DNA in their diseased tissues, whereas mutated k-ras sequence was not detected in the diseased tissues of patients 7–12.

<sup>b</sup> (+), DNA sample contains detectable mutated k-ras DNA in the RE-PCR assay; (−), DNA sample does not contain detectable mutated k-ras codon 12 DNA in the RE-PCR assay.

<sup>c</sup> CRC, colorectal cancer; Admn, adenomatous.

<sup>d</sup> The limit of detection is 0.3 µg/L, or 0.015 ng/50 µL.

To investigate this possibility, we isolated DNA from commercially available human serum (Sigma) by the two different methods. The DNA was then separated by polyacrylamide gel electrophoresis and stained with ethidium bromide, as shown in Fig. 1. As expected, the serum contained DNA fragments the size of nucleosomal DNA, which were resolved in an 8% polyacrylamide gel (as indicated by the arrows in Fig. 1), and larger DNA, which remained near the sample wells of the gel. Interestingly, much smaller amounts of the low-molecular-weight DNA were observed when DNA was isolated by the Qiagen method (Fig. 1, lane 4). The only species that was detected was DNA in the size of mononucleosomes. DNA fragments that were in the size of di- and trimucleosomes were not seen. The most abundant DNA detected in the Qiagen preparation was the high-molecular-weight DNA, which remained unresolved and near the sample wells in the gel. In contrast, DNA in the size of mono-, di-, and trimucleosomes was readily detected in the serum DNA isolated by the G/R method (Fig. 1, lanes 2 and 3) when DNA was resolved in the gel, with very little high-molecular-weight DNA remaining near the well. There was less high-molecular-weight DNA remaining near the sample wells even when DNA was isolated from three times more serum by the G/R method (Fig. 1, lane 3) compared with the DNA isolated by the Qiagen method (Fig. 1, lane 4). These data clearly demonstrate that the DNA isolated by the Qiagen method was enriched in higher molecular weight DNA that remained unresolved in an 8% polyacrylamide gel, whereas the DNA isolated by the G/R method was enriched in shorter, fragmented DNA.

It has been suggested that there are two categories of circulating DNA (3). One is large, genomic DNA, mostly derived from necrotic or hematopoietic cells; the other

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![Fig. 1. Electrophoresis of serum DNA isolated by the Qiagen and G/R methods.](image-url)

DNA isolated from 2 mL (lane 2) or 6 mL (lane 3) of serum by the G/R method or from 2 mL (lane 4) of serum by the Qiagen method was resolved by electrophoresis in an 8% polyacrylamide gel. The gel was stained with ethidium bromide and photographed under ultraviolet illumination. Lanes 1 and 5 are molecular size markers. The DNA fragments the size of mononucleosomes (mono), dinucleosomes (di), and trimucleosomes (tri) are indicated by arrows. These data represent the results from three independent experiments.
species is fragmented, cell-free DNA the size of nucleosomal DNA, mostly derived from cells undergoing apoptosis. As discussed earlier, the source of mutated k-ras DNA in the circulation could be apoptotic cells. Thus, mutated k-ras DNA should be enriched in the fragmented, nucleosomal DNA species relative to the large species found near the sample wells of the gel. Therefore, the DNA isolation method that favors isolation of fragmented DNA should produce a better ratio of k-ras-mutated DNA to wild-type DNA. As shown in Table 1, mutated k-ras was readily detected in the DNA isolated by the G/R method but was detected less frequently in DNA isolated by the Qiagen method from the specimen from the same patient, although more DNA was recovered by the Qiagen method. Another possible contributor to this discrepancy in assay results is the nature of the RE-PCR assay. This is a PCR-based assay, and with more wild-type genomic DNA in the assay, the sensitivity of detection of the mutated DNA is reduced because of interference with the PCR.

This study clearly demonstrates that the method chosen for isolation of DNA can contribute significantly to the outcome of mutation detection (in this case, k-ras mutations). It has been suggested that malignant, benign, and even preneoplastic cells often proliferate at abnormal rates, accompanied by an increase in apoptotic cell death (9–11), and that this small, fragmented DNA may accumulate in the circulation. These results further suggest that to enhance assay sensitivity for detection of somatic mutations or epigenetic modifications in circulating DNA for cancer detection, monitoring, or prognosis, a method that can preferentially isolate small DNA should be used.

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Plasma Mitochondrial DNA Concentrations after Trauma, Nicole Y.L. Lam,1 Timothy H. Rainer,1 Rossa W.K. Chiu,2 Gavin M. Joynt,3 and Y.M. Dennis Lo* (1 Accident and Emergency Medicine Academic Unit, 2 Department of Chemical Pathology, and 3 Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR; * address correspondence to this author at: Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Room 38023, 1/F Clinical Sciences Bldg., 30-32 Ngan Shing St., Shatin, New Territories, Hong Kong SAR; fax 852-2194-6171, e-mail loyym@cuhk.edu.hk)

Both qualitative and quantitative studies on circulating DNA (1–3) and RNA (4–6) in plasma and serum have shown their usefulness in clinical diagnosis and prognosis. In addition to DNA and RNA, there is another species of nucleic acids, mitochondrial DNA (mtDNA), that is usually confined to and functions in the mitochondria. Mutations in the mitochondrial genome are associated with various diseases (7). A recent study reported the presence of a known mtDNA mutation in the serum and plasma of patients with type II diabetes mellitus (8). Mutant mtDNA has been detected in plasma of patients with hepatocellular carcinoma (9). However, unlike circulating DNA, there is a paucity of quantitative data on circulating mtDNA. The reason might be partly attributable to the presence of pseudogenes in the nuclear genome that may lead to coamplification of these nuclear copies of mtDNA (10). To overcome this problem, a mtDNA-specific real-time quantitative PCR assay has recently been developed (11).

Significant increases in circulating DNA in the plasma of trauma patients have been reported and found to be useful in posttraumatic prognosis (12). One hypothesis for the increased plasma DNA concentration after trauma is that cell-free DNA is released from damaged tissues to the nearby bloodstream. We therefore proposed that...