Agreement between K-ras Sequence Variations Detected in Plasma and Tissue DNA in Pancreatic and Colorectal Cancer

To the Editor:
Several studies have identified DNA alterations in circulating plasma DNA of cancer patients that match genetic changes in primary tumors, but the low sensitivity obtained has limited the clinical application of plasma analysis (1, 2). A recent report in Clinical Chemistry (3) demonstrated that the method chosen to isolate plasma DNA, a modified guanidine/Promega resin (G/R) method, could increase detection of K-ras sequence variations in patients with colorectal disease. Using the same approach, another group (4) found no relationship between these isolation methods, nor between K-ras sequence variations found in DNA from plasma and tumor tissue in patients with non-small cell lung cancer. In a previous study (5), we found that plasma K-ras analysis was a highly specific, low-sensitivity approach with prognostic significance in pancreatic carcinoma.

To evaluate the agreement rate between K-ras sequence variations in plasma DNA and corresponding tissue, we used the Qiagen method to isolate DNA from 112 plasma samples from patients with pancreatic disease and from 87 plasma samples from patients with colorectal disease. We also isolated DNA from corresponding pancreatic cytology samples and colorectal tissues. The restriction fragment length polymorphism–PCR method used to detect K-ras sequence variations has been described previously (6). The concordant results between plasma and tissue are shown in Table 1.

In the patients with pancreatic adenocarcinoma, sensitivity for detecting K-ras sequence variations was 43% (19 of 44) in plasma samples and 87% (39 of 45) in fine-needle aspirate or pancreatic juice samples. No sequence variations were detected in plasma DNA from patients with chronic pancreatitis, acute pancreatitis, or other pancreatic neoplasms, giving a specificity of 100%. The agreement rate in pancreatic samples was 78% (19 positive and 67 negative; total, 86 of 110). Single-strand conformation polymorphism (SSCP) analysis allowed characterization of 11 of 19 positive plasma samples, and the spectrum was 8 GAT and 3 GTT. Concordant SSCP results were obtained in plasma and cytology samples. In the colorectal adenocarcinoma group, sensitivity for detecting K-ras sequence variations was 8.5% (7 of 82) in plasma samples and 41% (34 of 82) in resected tissue samples. In colorectal adenomas and diverticulosis, no variant sequences were detected in plasma or tissue. The agreement rate in colorectal plasma and tissue samples was 69% (7 positive and 53 negative; total, 60 of 87). Characterization was possible in 5 of 7 positive plasma samples (3 GAT, 1 GTT, and 1 CGT) and was concordant with results in tissue.

In addition, we performed our routine Qiagen assay method in parallel with the G/R method in a subset of 12 plasma samples (6 pancreatic and 6 colorectal adenocarcinomas) from the evaluated group (3 with the K-ras variant in each group). With the G/R

Table 1. Agreement rate between plasma and pancreatic or colorectal tissue.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>Plasma</th>
<th>Pancreatic (FNA or PJ) or colorectal tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic adenocarcinomas</td>
<td>45</td>
<td>19 (+)</td>
<td>19 (+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 (+)</td>
<td>5 (-)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (NA)</td>
<td>1 (-)</td>
</tr>
<tr>
<td>Other pancreatic neoplasms</td>
<td>8</td>
<td>8 (-)</td>
<td>8 (-)</td>
</tr>
<tr>
<td>Chronic pancreatitis</td>
<td>53</td>
<td>52 (-)</td>
<td>48 (-)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (NA)</td>
<td>1 (-)</td>
</tr>
<tr>
<td>Acute pancreatitis</td>
<td>6</td>
<td>6 (-)</td>
<td>6 (-)</td>
</tr>
<tr>
<td>Colorectal adenocarcinomas</td>
<td>82</td>
<td>7 (+)</td>
<td>7 (+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75 (-)</td>
<td>48 (-)</td>
</tr>
<tr>
<td>Colorectal adenomas</td>
<td>3</td>
<td>3 (-)</td>
<td>3 (-)</td>
</tr>
<tr>
<td>Diverticulosis</td>
<td>2</td>
<td>2 (-)</td>
<td>2 (-)</td>
</tr>
</tbody>
</table>

* (+): DNA sample contains detectable codon 12 K-ras mutation; (-), DNA sample does not contain detectable codon 12 K-ras mutation.

a FNA, fine needle aspirate; PJ, pancreatic juice.

b NA, nonamplified samples (in 2 plasma pancreatic samples amplification was not possible).

c Including 3 cholangiocarcinomas, 3 pancreatic metastases, and 2 neuroendocrine tumors.
method, we could not improve K-ras detection, but the intensity of the 143-bp band depicting the mutant allele (6) was higher than that obtained with Qiagen.

In conclusion, our results support the relationship between K-ras sequence variations detected in DNA from plasma and tumor tissue in pancreatic and colorectal cancer. Although plasma analysis is a low-sensitivity approach, it may be a confirmatory tool when a more invasive diagnostic technique is contraindicated. The G/R method could offer a valuable alternative method for plasma DNA isolation.

References


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