**Methylation of BCAT1 and IKZF1 DNA in tissue and plasma from colorectal cancer cases**

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**BACKGROUND**

We have previously described a 2-gene blood test based on the detection of methylated BCAT1 and IKZF1 for colorectal cancer (CRC) detection. This study explored the relationship between the presence of these methylated DNA biomarkers in plasma and tissue from CRC patients.

**STUDY SYNOPSIS**

Objectives

To compare the levels of methylated BCAT1 and IKZF1 DNA in blood and tissue samples from CRC patients.

Study Design

An observational study collecting blood pre-resection and colon tissue samples from patients diagnosed with CRC.

Study Cohort

75 patients undergoing initial surgery for CRC (median age 64 years (range 44-85) 49.3% male).

Methods

K3EDTA-blood was collected prior to surgery on patients diagnosed with CRC. Tumor and tissue samples were collected from patients who had been diagnosed with CRC. DNA in matched CRC and normal adjacent tissue samples. Boxwhisker bars:

- Tissue CRC negative (n = 1)
- Plasma negative (n = 1)
- Tissue CRC positive only (n = 1)
- Plasma positive only (n = 1)
- Tissue and Plasma positive (n = 1)

*ANOVA p <0.05. **Chi2 p-value <0.05. Symbols: mean, horizontal bars: 95%CI.

Figure 1. Methylation levels in 75 matched tissue and plasma samples (A) % methylated BCAT1 (B) and plasma samples (C). The level of methylated IKZF1 DNA in CRC tissue and plasma samples was assessed as previously described (1–2), and expressed as a percentage of either 5 ng tissue DNA or of total cell-free DNA recovered from 4 mL plasma. Positivity rate was expressed as the proportion of cases with any detectable signal of methylated BCAT1 and/or IKZF1.

Table 1. Methylation of BCAT1 and IKZF1 in tissue and blood samples

<table>
<thead>
<tr>
<th></th>
<th>BCAT1</th>
<th>IKZF1</th>
<th>Combined</th>
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<tbody>
<tr>
<td>Tissue</td>
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<td>Plasma</td>
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<tr>
<td>Tumor tissues</td>
<td>68 (91%)</td>
<td>69 (92%)</td>
<td>74 (99%, 95%CI: 93-100)</td>
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<tr>
<td>Plasma samples</td>
<td>35 (47%)</td>
<td>36 (48%)</td>
<td>48 (64%, 95%CI: 52-75)</td>
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</tbody>
</table>

Figure 2. Quantitative Venn Diagram illustrating methylation positivity

Figure 3. BCAT1 and IKZF1 methylation levels in tissue and morphological subtypes

Bioinformatically-converted DNA extracted from matched samples (75) including normal adjacent tissue (A), CRC tissue (B) and plasma samples (C). The level of methylated BCAT1 and IKZF1 DNA was expressed as the combined %methylated BCAT1 + IKZF1 measured in 5 ng tissue DNA (A/B) or total amount of DNA from 4 ml plasma (A/C). The number of plasma samples positive for methylated BCAT1 is also shown.

Figure 4. Methylation levels in matched samples

For one metastatic case, primary colon tumor, liver metastases and blood samples were available and tested for methylated BCAT1 and IKZF1. BCAT1/IKZF1 methylation in tissue is expressed as combined %methylated per 5 ng total tissue DNA. Blood any methylation signal = positive.

**RESULTS**

- Methylated BCAT1 and/or IKZF1 were detected in 74/75 (99%) tumor tissue samples. Combined methylation levels were significantly higher in CRC than in normal adjacent tissue samples (5th methylated DNA: 47.67 vs. 3% (2.7–4.6) p <0.001, Table 1, Fig 2).
- In matching plasma samples, methylated BCAT1 and/or IKZF1 DNA was detected in 35 and 36 samples, respectively, with a total of 48 of the 75 (64%) samples being positive for at least one of the biomarkers, Table 1 and Fig 2.
- The concentration of methylated BCAT1 and IKZF1 DNA in plasma was not strongly dependent upon tissue levels, although a positive trend was observed between appearance of the methylated biomarkers in plasma and degree of tumor invasiveness, Fig 3B and 3C. The one tumor tissue sample negative for BCAT1/IKZF1 methylation was also negative in plasma.
- Tissue methylation levels were independent of morphology, whereas the levels of the biomarkers in plasma were associated with depth of invasion and lymph node invasion. There was no association with tumor size, differentiation or lymphovascular invasion, Figs 3.
- For one metastatic case, primary tumor plasma and liver samples were available and analyzed, which confirmed methylated BCAT1/IKZF1 in all samples. Fig 4: No methylation was detected in adjacent non-cancer liver tissue.

**CONCLUSION**

- The high levels of methylated BCAT1 and IKZF1 in CRC tissue, regardless of morphology, and the low levels in the surrounding non-cancer tissue suggests that the markers are tumor specific with no obvious field effect.
- Appearance of the methylated biomarkers in blood depends on the primary tumor displaying invasive morphological features indicative of the tumor having access to the blood stream and shedding DNA into circulation.

**References**