The relationship between the degree of aberrant methylation in colorectal cancer tissue and appearance of tumor-derived DNA in blood

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BACKGROUND
We have previously described a blood test for colorectal cancer (CRC) based on the detection of methylated BCAT1 and IKZF1 in circulating tumor DNA (ctDNA)1-6. This study compared the levels of methylated BCAT1 and IKZF1 DNA in colon tissue with the levels measured in blood.

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, tumor and adjacent non-cancer tissue from CRC patients who did not receive neoadjuvant therapy.

Study Cohort: 189 diagnosed CRC patients with blood collection prior to resection

Methods: Tumor and adjacent non-CRC tissue samples were collected during surgery. Clinical histopathology reports were assessed to determine tumor characteristics. Blood samples were obtained before and after resection (no more than 12 months after conclusion of primary treatment). The levels of methylated IKZF1 and BCAT1 DNA in tissue were expressed as the percentage of 5 ng tissue DNA. Calculation of positivity rates: tissue, the proportion of tissue cases with 10% or more methylation; blood, the proportion of cases with any detectable signal.

RESULTS

Disposition and outcome of study cohort: 79 CRC patients (53.2% males, median age 70.0 years) including 14 Stage I, 31 Stage II, 26 Stage III, 8 Stage IV, with 44 post-operative plasma samples, Fig. 1.

Methylation levels in tissue: The methylation levels of BCAT1 and IKZF1 in colorectal tumors (BCAT1 49.1%, IKZF1 61.4%, Mann-Whitney p = 0.103) were significantly higher than the levels measured in adjacent non-CRC tissue (p-values <0.0001), Fig. 2. At a cutoff of 10% methylation, 79 (88.6%) and 62 (78.5%) of the 79 tumors were positive for BCAT1 and IKZF1 respectively (p = 0.085), with 93.7% being positive for either marker. In the adjacent non-CRC tissues, 16 (20.3%) were BCAT1 methylation positive only (>10% methylation, p = 0.0001).

Circulating tumor DNA (ctDNA): In the 79 matching plasma samples, 38 (48.1%) were positive for either BCAT1 or IKZF1 with either being positive in 63.3% (50/79).

Tissue methylation versus ctDNA: In the 79 matching plasma samples, 38 (48.1%) were positive for either BCAT1 or IKZF1, with either being positive in 63.3% (50/79). The tissue methylation levels did not differ across CRC stages (1-way ANOVA Stage II vs IV: tumor: p = 0.4911; normal: p = 0.8714), whereas the detection of ctDNA in blood was significantly associated with tumor staging (p = 0.0063), Table 1. Test concordance between CRC tissue and blood is shown in Figure 3. A significant discordance between CRC tissues and matched blood was only observed in those with early stage cancer (Stage I and II). Five tumors were deemed negative (methylation <10%), with three of these cases also having a negative matching plasma sample. The two positive plasma samples had a single PCR replicate being BCAT1 positive late in the real-time detection cycle.

ctDNA before and after resection: A post-operative blood sample was available for 23 of the 50 cases with a positive ctDNA result at initial diagnosis (2 Stage I, 11 Stage II, 8 Stage III and 2 Stage IV) and for 21 of the 29 cases with a negative blood result at diagnosis (10 Stage I, 7 Stage II, 4 Stage III). Of the 23 ctDNA positive cases at diagnosis, 18 (78%) were negative for ctDNA post resection, Fig 4. Residual disease was identified in the liver for the two of the four (40%) cases that remained ctDNA positive after resection and two cases tested ctDNA negative at a repeat blood test 6 months later. No further information was available for the other case that remained ctDNA positive post resection.

CONCLUSION
Aberrant BCAT1 and IKZF1 methylation is an early event in CRC development and is localised to the tumor tissue. Methylated BCAT1 and IKZF1 in blood are dependent on tumor stage. Measuring minimal residual disease by detecting ctDNA based on methylated BCAT1 and IKZF1 may inform completeness of tumor resection.