Methylated *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) (1). 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 adjacent non-cancer tissue (NCT) samples were collected during surgery. An expert pathologist assessed histopathology reports to determine cancer stage and characteristics (e.g. TNM, degree of differentiation, perivascular or perineurial invasion). The levels of methylated *IKZF1* and *BCAT1* DNA in blood and tissue samples were assessed as previously described (1-2), and expressed as a percentage of either 5ng tissue DNA or of total cell-free DNA recovered from 4mL plasma. Positivity rate was expressed as the proportion of cases with any detectable signal of methylated *BCAT1* and/or *IKZF1*.

Figure 1. Methylation levels in 75 matched tissue and plasma samples

(A) % methylated *BCAT1* and *IKZF1* DNA in matched CRC and normal adjacent tissue samples. Boxwhisker bars: 95% CI. Mean: + symbol, Median: horizontal bar. (B) Correlation plot for %methylated *BCAT1/IKZF1* DNA measured in CRC tissue (x-axis) versus plasma samples (y-axis).

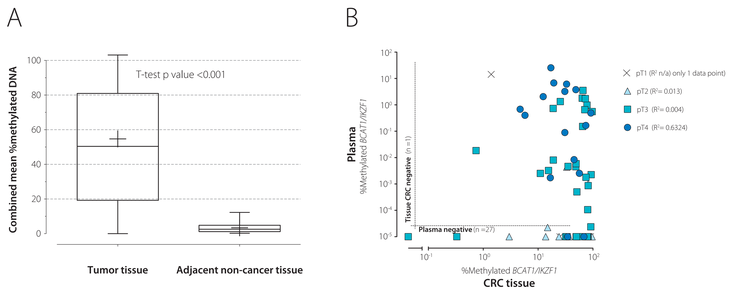


Table 1. Methylated *BCAT1* and *IKZF1* positive counts in blood and tissue

n = 75	*BCAT1*	*IKZF1*	Combined
Tumor tissues	68 (91%)	69 (92%)	**74** (99%, 95%CI: 93-100)
Plasma samples	35 (47%)	36 (48%)	**48** (64%, 95%CI: 52-75)

Figure 2. Quantitative Venn Diagram illustrating methylation positivity

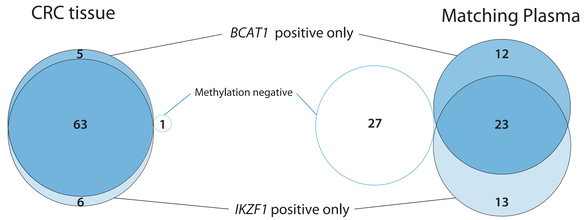
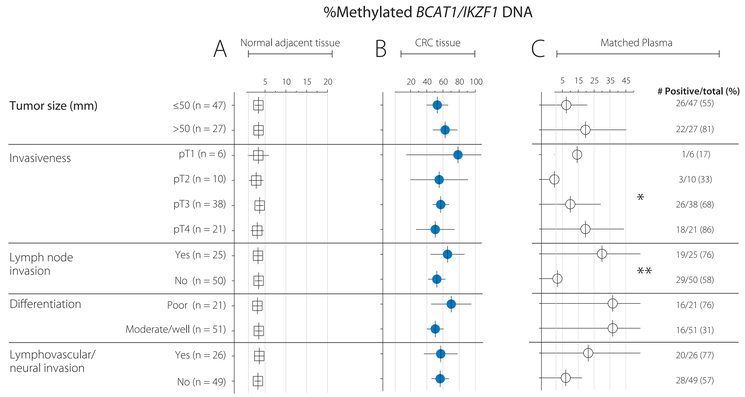


Figure 3. *BCAT1* and *IKZF1* methylation levels and tumor morphology

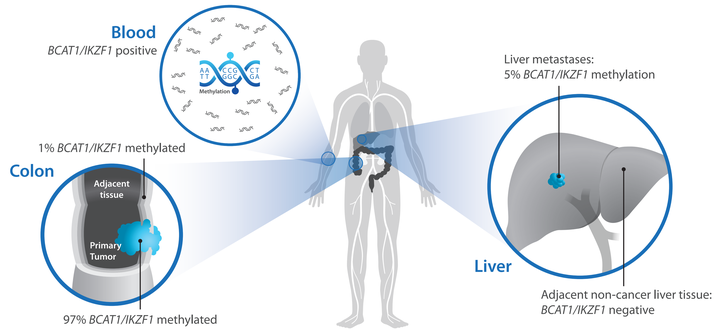
Bisulphite-converted DNA extracted from matched samples (75) including normal adjacent tissue (A), CRC tissue (B) and plasma samples (C). The level of methylated *BCAT1* and *IKZF1* DNA was expressed as the combined %methylated *BCAT1* + *IKZF1* measured in 5ng tissue DNA (A,B) or total amount of DNA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



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

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 5ng 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 (56% methylated DNA (95%CI: 47-67) versus 3% (2.7-4) (p <0.001), Table 1, Fig 1A.
- In matching plasma samples, methylated *BCAT1* and *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 1B 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 lymphovascularity, Fig 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.

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References: **1.** Pedersen *et al. BMC Cancer* 2015;15. (2):654-. **2.** Mitchell *et al. BMC Cancer* 2014;14;54-