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

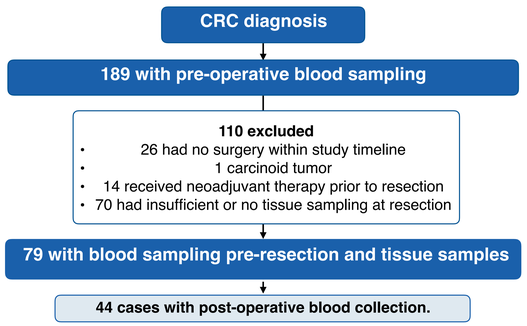
Objectives: To compare the levels of methylated *BCAT1* and *IKZF1* DNA in blood and tissue samples from CRC patients.

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% methylation; blood, the proportion of cases with any detectable signal of methylated *BCAT1* and/or *IKZF1*.

Figure 1. Disposition & outcomes of study cohort



References: (1) Mitchell et al. BMC Cancer 2014; (2) Pedersen et al. BMC Cancer 2015; (3) Symonds et al CTG 2016; (4) Pedersen et al. PLoS One 2015; (5) Symonds et al. APJCP 2015;(6) Young et al. Cancer Medicine 2016 .

Table 1. Methylation in matched CRC and normal adjacent tissue and plasma samples

		CRC tissue				Adjacent non-CRC tissue				Matched pre-operative plasma			
		Positivity			%Methylation[1]	Positivity			%Methylation	Positivity			pg/mL ctDNA[2]
	N	n	%	95%CI	Median (IQR)	n	%	95%CI	Median (IQR)	n	%	95%CI	mean (median, IQR)
All	79	74	**94**	86-98	50 (24-79)	16	**20**	12-31	3 (1-5)	50	**63**	51-74	385 (2.2, 0-18)
I	14	13	**93**	66-100	50 (22-92)	2	**14**	2-43	3 (1-5)	2	**14**	2-43	7 (0, na)[3]
II	31	28	**90**	74-98	42 (17-81)	6	**19**	7-37	2 (1-4)	21	**68**	49-83	33 (3.3, 0-20)
III	26	25	**96**	80-100	52 (33-74)	5	**19**	7-39	3 (1-5)	20	**77**	56-91	101 (2.3, 0-18)
IV	8	8	**100**	63-100	45 (19-103)	3	**38**	9-76	7 (2-5)	7	**88**	47-100	4250 (205, 7-24752)

[1]Median expression levels including all cases in a row category (N); IQR: interquartile range; [2]The 2-gene real-time PCR assay is only quantitative down to ~120pg/mL, hence calculated concentrations below this are extrapolated and most likely inaccurate. [3]Median mass from the two positives was 0.

Figure 2. Tissue methylation levels

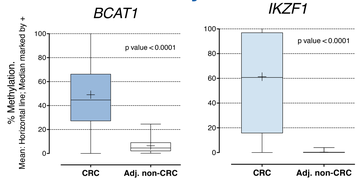


Figure 3. Concordance of *BCAT1/IKZF1* positivity rates in matched tissue and plasma

Early stage CRC

14 cases with Stage I		CRC tissue +	CRC tissue -
Blood	+	**2**	**0**
	-	**11**	**1**

McNemar's p value: 0.001

31 cases with Stage II		CRC tissue +	CRC tissue -
Blood	+	**20**	**1**
	-	**8**	**2**

McNemar's p value: 0.039

Late stage CRC

26 cases with Stage III		CRC tissue +	CRC tissue -
Blood	+	**19**	**1**
	-	**6**	**0**

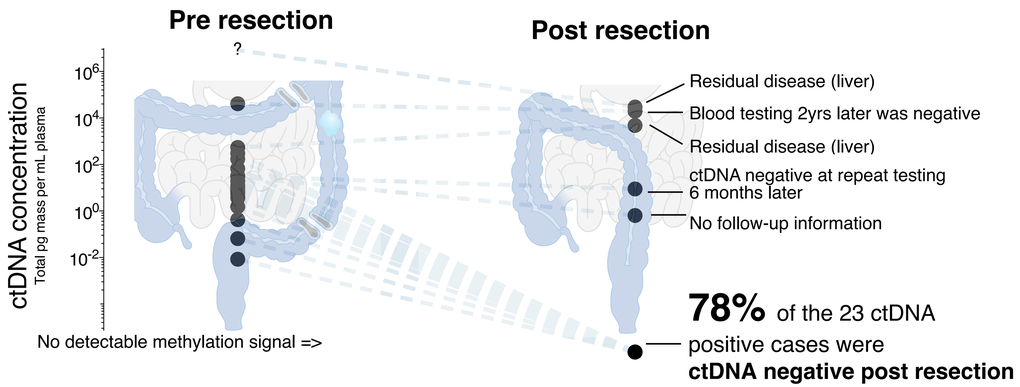
McNemar's p value: 0.125

8 cases with Stage IV		CRC tissue +	CRC tissue -
Blood	+	**7**	**0**
	-	**1**	**0**

McNemar's p value: 1.00

Figure 4. ctDNA levels before and after resection

The pre-resection sample marked "?" was IKZF1 positive but the amount of methylation was unknown



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, 70 (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 blood: The tissue methylation levels did not differ across CRC stages (1way ANOVA Stage I to IV): tumor, p = 0.4911; normal, p = 0.9714), 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 diagnoses (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 two of the five (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.

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