Specificity of methylated *BCAT1* and *IKZF1* for colorectal cancer

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BACKGROUND

Aberrant methylation plays an important role in tumor development. Methylated *BCAT1* and *IKZF1* are useful circulating tumor DNA (ctDNA) biomarkers for detection and following the course of colorectal cancer (CRC).[1-3] It is not clear whether other invasive adenocarcinomas, such as breast, oesophageal or prostate cancers, are also associated with the presence of ctDNA methylated in *BCAT1* and/or *IKZF1*.

AIM

This study aimed to determine the specificity of methylated *BCAT1* and *IKZF1* for CRC detection by assaying blood and tissue specimens from patients with other adenocarcinomas.

STUDY COHORT

An observational study was performed which collected blood from patients with invasive adenocarcinoma of the prostate (n=32), breast (16), oesophagus (20) or colon/rectum (211), prior to any treatment or resection, and from 245 clinically assessed controls with no known prior or current adenocarcinoma, Table 1. In addition, biopsies were collected from cancer and adjacent non-neoplastic tissues either prior to treatment or at surgery from patients with prostate (n = 9), breast (26), oesophageal (6) and colon/rectum (15) cancer.

METHODS

DNA was extracted from 4 mL K_3EDTA plasma and 10-20mg tissue and bisulphite converted. All DNA samples were assayed for methylated *BCAT1* and *IKZF1* DNA using a real-time multiplex PCR assay previously described.[3] A total of 5ng bisulphite converted tissue DNA was assayed for methylation in *BCAT1* and *IKZF1* and the level of expression was expressed as the percentage of the total input (determined with *ACTB*). Tissue samples were considered positive if the %methylation of either gene was above the 75th percentile of non-neoplastic tissue levels. Blood samples were considered positive with any detectable signal of either methylation marker.

Table 1. Study cohort

Group	No. male (%)	Age (median, IQR), years
Control	92/253 (36.4%)	57.70 (51.25-65.20)
Colorectal cancer	133/211 (62.6%)	68.60 (60.50-76.60)*
Oesophageal cancer	17/20 (85%)	70.50 (56.08-76.03)*
Prostate cancer	33/33 (100%)	67.80 (59.85-73.30)*
Breast cancer	0/16 (0%)	61.80 (48.63-71.78)

* p<0.05 compared to Control group

Funding

Funding was provided by a seeding grant from the Flinders Medical centre (FMC) Foundation (2015).

Figure 1. *BCAT1* & *IKZF1* methylation levels in tumor and adjacent non-neoplastic tissues. * p < 0.05; ** p < 0.001

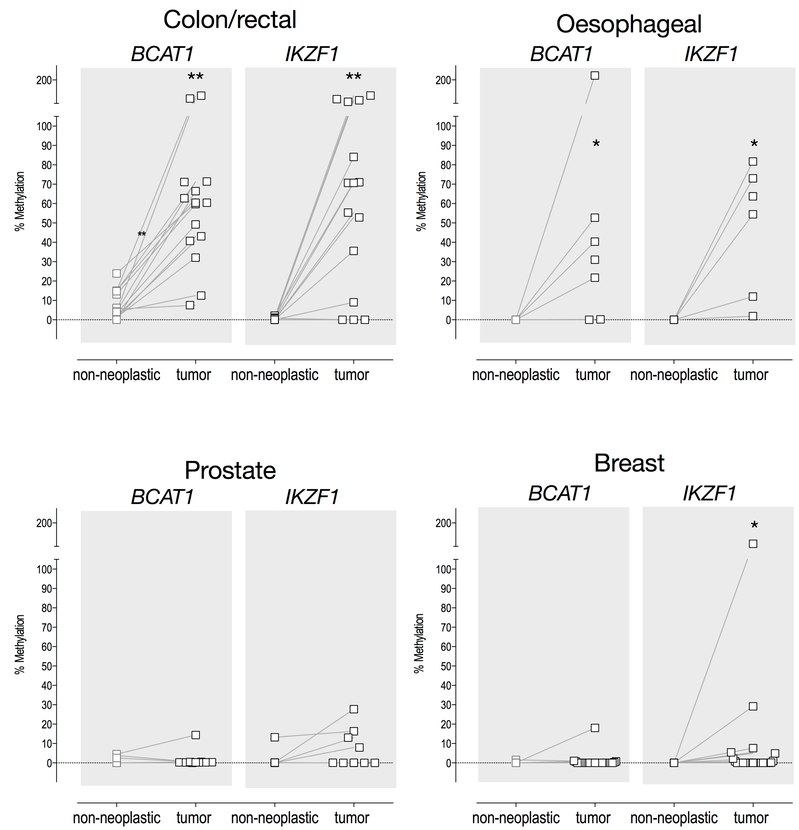

Table 2. Tissue methylation levels

Cancer Type	N	Non-neoplastic tissue, median (IQR)		Tumor tissue, median (IQR)	
		BCAT1	*IKZF1*	*BCAT1*	*IKZF1*
Colorectal	15	4.2 (2.6-13.9)	0 (0-0.7)	60.4 (41.9-71.3)	70.7 (22.3-116.1)
Oesophageal	6	0 (0-0)	0 (0-0)	31.0 (5.6-49.6)	63.7 (22.6-79.5)
Prostate	9	0 (0-2.5)	0 (0-0.2)	0.3 (0.2-0.5)	0.1 (0-13.0)
Breast	26	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0.95)

Acknowledgements

Tissue specimens were kindly provided by the Flinders Tumor Bank. We thank the involvement in patient recruitment of the Department of Surgery at Flinders University, the Flinders Breast Cancer Unit at Flinders Medical Centre, and the Urology Department at Repatriation General Hospital.

Table 3. Blood positivity in early v. late stage cancers

Cancer Type	Positivity Rate, Number of Positive/ Total (%)		P-value[1]	Positivity rate, Number of Positive/Total (%) - all cases
	Early stage	Late stage		
Control	not applicable	not applicable	N/a	16/245 (6.5%)
Colorectal	44/107 (41.1)	82/104 (78.8)	<0.01	126/211 (59.7%)[3]
Oesophageal[2]	1/6 (16.7)	8/13 (61.5)	0.07	10/20 (50%)[3]
Prostate	2/23 (8.7)	2/9 (22.2)	0.557	4/32 (12.5%)
Breast	2/14 (14.3)	0/2 (0)	1.00	2/16 (12.5%)

[1] Relative to early stage; [2] One oesophageal cancer was unstaged; [3] p < 0.01 compared to control

RESULTS

TISSUE

- CRC and oesophageal cancer tissues had significantly higher methylation levels compared to matched non-neoplastic tissue for both *BCAT1* and *IKZF1*, Figure 1 (top panel)
- The median methylation levels in prostate (*BCAT1*, 0.3%; *IKZF1*, 0.1%) and breast (0%) cancer tissues were 2-3 orders of magnitude less than the levels measured in CRC (60.4%; 70.7%) and oesophageal (31.0; 63.7%) cancer tissues, Table 1 & Figure 1 (bottom panel).
- Using the 75th percentiles measured in non-neoplastic tissues as positivity cutoffs, the cancer tissue positivity rates were: CRC, 14/15, 93.3% (95%CI: 68.1-99.8); oesophageal, 6/6, 100% (54.1-100); prostate, 4/9, 44.4% (13.7-78.8); breast, 11/26, 42.3% (23.4-63.1).

BLOOD

- ctDNA positivity rates were significantly higher in CRC (126/211, 59.7%, 95% CI: 52.8 - 66.4) and oesophageal cancer (10/20, 50%, 27.2-72.8) cases only compared to controls (16/245, 6.5%, 3.8 - 10.4; p < 0.01).
- ctDNA was more likely to be positive in late stage cancers, although only significant for CRC, Table 2. There were no significant associations of the other cancers and positivity status with any patient demographics or tumor features (p > 0.05).

CONCLUSION

- Only colorectal and oesophageal cancer patients had significantly higher ctDNA positivity rates (using methylated *BCAT1/IKZF1*) compared to controls. This was also reflected in a higher proportion of cases showing methylation in the cancer tissue.
- The methylated *BCAT1/IKZF1* blood test should be investigated further as a screening and surveillance tool for oesophageal cancer, as well as its existing use for CRC.

References

1. Pedersen SK, et al. PLoS One 2015
2. Pedersen SK, et al. BMC Cancer 2015
3. Symonds EL, et al Clin Transl Gastro 2016