CLINICAL EVALUATION OF A NOVEL 2-GENE METHYLATION BLOOD TEST FOR COLORECTAL CANCER

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BACKGROUND

A blood test for colorectal neoplasia may improve participation rates and may be more specific for neoplasia-associated molecular changes than FOBT/FIT. We have undertaken a 5-year biomarker program using microarrays and bisulphite deep sequencing to identify candidate DNA methylation biomarkers for colorectal neoplasia.

AIM

To gather preliminary data on the clinical utility of a novel two-gene methylation test for detection of both colorectal carcinoma and adenomas testing DNA extracted from tissue and blood plasma.

MATERIALS and METHODS

- Methylation-specific PCR (MSP) assays were designed (Table 1) for initial validation in neoplastic and control tissues.
- DNA was extracted from colorectal tissues using a Wizard Genomic DNA purification Kit (Promega) and 1ug bisulphite-converted using EZ Bisulfite DNA Methylation Gold (Zymo). 5ng DNA was used to validate MSP assays.
- Circulating DNA was extracted from 4mL plasma from colonoscopy-confirmed patients using QiaAmp Circulating Nucleic Acid kit (Qiagen) and bisulphite-converted using Epitect Plus bisulphite kits (Qiagen).
- MSP assays were performed in triplicate using the equivalent of 0.5mL plasma per replicate.
- Two MSP assays were evaluated in an independent cohort of 251 colonoscopy-confirmed patient plasma specimens including a mix of retrospectively collected (155) and prospectively collected (96) case control specimens.
- All validation experiments were blinded to operator.

RESULTS

Candidate loci show hypermethylation in cancer tissue, Figure 1.

- Two markers, BCAT1 (branched-chain aminotransferase 1) and IKZF1 (Ikaros family zinc-finger 1), showed high levels of methylated DNA in cancer and adenoma tissue, compared to normal tissue

BCAT1 and IKZF1 methylation in clinical samples, Figures 2 and 3

- BCAT1 and IKZF1 methylation was evaluated in an expanded clinical cohort of 251 case control plasma specimens (74 cancers, 33 adenomas, 144 normals) and showed high sensitivity for cancers, lower sensitivity for adenomas, and little or no methylated DNA in normal plasma.

BCAT1 and IKZF1 are generally co-methylated, Figure 4

- While BCAT1 and IKZF1 methylation correlated in most samples, some samples show methylation at only one locus

BCAT1 and IKZF1 two-gene test summary, Figure 5

- A BCAT1/IKZF1 two-gene methylation test detects 76% of cancers with few false positives (7%). The test has better sensitivity for late-stage rather than early-stage cancers.

CONCLUSIONS and FUTURE DIRECTIONS

- A two-gene methylation test for BCAT1 and IKZF1 detects 76% of cancers with 93% specificity.
- Two expanded clinical trials are now underway to evaluate this test relative to established screening methods such as FIT and colonoscopy.

TABLE I

MSP assay details

BCAT1

BCAT1 Forward: 5' GTTTTTTTGTTGATGTAATTCGTTAGGTC
BCAT1 Reverse: 5' CAATACCCGAAACGACGACG
BCAT1 Probe: HEX-5' TTCGTCGCGAGAGGGTCGGTT -BHQ1

Assay conditions: Platinum Taq (Invitrogen; 0.033U/uL) with 4mM MgCl2, 200uM dNTPs, 200nM primers and 100nM Probe. 5uL template into 15uL final PCR volume.
Cycling conditions: 1 cycle 95°C/2 min; 50 cycles 95°C/15sec, 62°C/30sec, 72°C/30sec

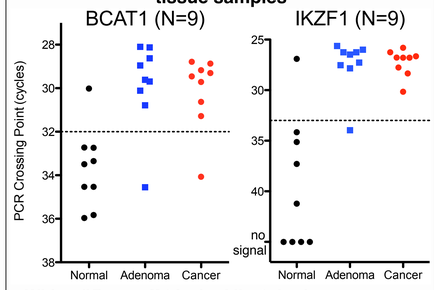
IKZF1

IKZF1 Forward: 5' GACGACGTATTTTTTTCGTGTTTC
IKZF1 Reverse: 5' GCGCACCTCTCGACCG
Detection: Sybr Green with melting-temperature analysis

Assay conditions: GoTaq Hot Start mastermix (Promega; 1X) with 4mM (total) MgCl2, 200nM primers and 0.001% Sybr Green (Molecular Probes). 5uL template into 15uL final PCR volume.
Cycling conditions: 1 cycle 95°C/2 min; 50 cycles 95°C/15sec, 62°C/30sec, 72°C/30sec; 1 cycle melt analysis (95°C/10sec, 65°C/1min, ramp to 97°C at 0.11°C/sec with continuous acquisition.

FIGURE 1

Validation of BCAT1 and IKZF1 MSP assays in tissue samples



With a CP cut-off (dashed line), both assays were positive in 8/9 or 9/9 cancers, 8/9 adenomas, but only 1/9 normal tissue samples

FIGURE 2

BCAT1 MSP assay in 251 blood plasma samples

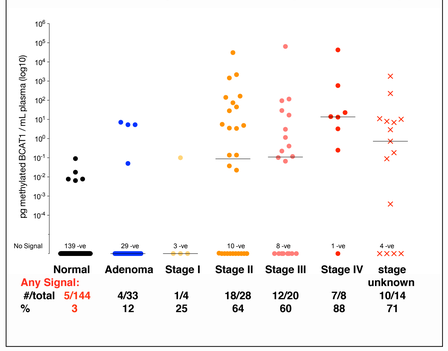


FIGURE 3

IKZF1 MSP assay in 251 blood plasma samples

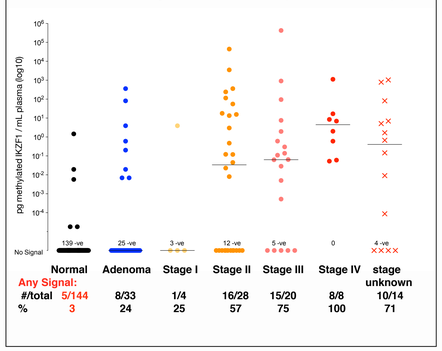


FIGURE 4

Correlation between BCAT1 and IKZF1 methylation

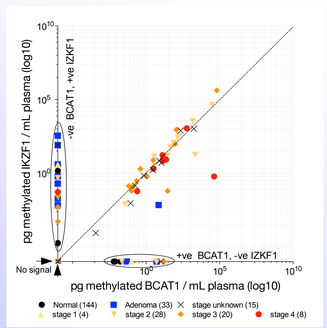


FIGURE 5

Two-gene test summary

SPECIMENS	BCAT1 +ve (%)	IKZF1 +ve (%)	2-GENE PANEL
144 Normals	5 (3%)	5 (3%)	10 (7%)
33 Adenomas	4 (12%)	8 (24%)	11 (33%)
4 Stage I	1 (25%)	1 (25%)	2 (50%)
28 Stage II	18 (64%)	16 (57%)	19 (68%)
20 Stage III	12 (60%)	15 (75%)	16 (80%)
8 Stage IV	7 (88%)	8 (100%)	8 (100%)
14 Unkn Stage	10 (71%)	10 (71%)	11 (79%)

93% Specificity

76% Sensitivity, any cancer
66% Sensitivity, Stage I+II
86% Sensitivity, Stage III+IV

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