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.

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