Case Control Study of Methylation in a 20-Marker Panel and Adenomatous Polyp Formation

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Purpose

- The aim of the present study was to test the hypotheses that individuals with adenomas presented different methylation patterns in peripheral blood leukocytes (PBLs) of 20 candidate markers and their potential roles of the carcinogenesis process (*APC*, *BRCA1*, *CDKN2AP16*, *CYP24A*, *CYP27B1*, *ER-alpha*, *IGF2*, *MGMT*, *MINT1*, *MLH1*, *NGFR*, *PER1*, *PER2*, *PER3*, *SEPT9*, *SFRP4*, *SFRP5*, *TIMP3*, *TMEFF2*, and *WIF1*) compared to individuals without adenomas.

Background & Significance

- Colonoscopies are often rife with cultural, socioeconomic, and geographic barriers → low compliance → higher burden in some groups
  - 62% of eligible adults have been screened for CRC
  - Need to develop screening methods that involve accessible samples
- Stool-based tests (fecal occult blood test [FOBT] and the fecal immunochemical test [FIT])
  - Poor sensitivity of premalignant lesions, e.g. adenomas (FOBT: 7-23%; FIT: 13-26%)

- Development of blood-based biomarkers (i.e., epigenetic markers)
- Epigenetic alterations have been observed at different stages in the adenoma-carcinoma sequence
  - Epigenetic changes in PBLs have the ability to reflect target tissues
- Identification of novel markers associated with adenoma risk may lead to a better understanding of carcinogenesis pathways
  - Clock genes, particularly *Period* (*PER)* genes
    - May play a role in tumorigenesis

Methods

- Epigenetics and Diet in the Carcinogenesis Process (EDCaP) study (n = 143)
  - 107 subjects with methylation data
- Cases (n = 38): individuals with at least one histologically confirmed adenoma
- Controls (n = 69): individuals with a normal colonoscopy, or a normal biopsy not requiring heightened surveillance (hyperplastic polyp)

- 20 candidate markers were selected on the basis of previous literature and their potential roles of the carcinogenesis process
  - Methylation assessed via methylation-specific PCR
- Covariates were extracted from interviews and medical records
- Unconditional logistic regression was used to estimate crude and adjusted odds ratios (OR) with 95% confidence intervals (95% CI)

Results

Table 2. Unadjusted and adjusted effects of methylation markers in blood on adenoma formation

Candidate Gene	Cases (N = 38) N (%)	Controls (N = 69) N (%)	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Hypomethylation						
APC[a]	23 (61)	44 (64)	0.87 (0.39 – 1.97)	0.74	1.02 (0.42 – 2.46)	0.97
BRCA1[b]	18 (47)	31 (44)	1.10 (0.50 – 2.44)	0.81	1.54 (0.62 – 3.86)	0.36
CYP24A[c]	0 (100)	0 (100)	1.00 (1.00 – 1.00)	1.00	1.00 (1.00 – 1.00)	1.00
MINT1[d]	7 (18)	4 (6)	3.67 (1.00 – 13.48)	0.05	5.33 (1.01 – 28.17)	0.05
PER1[e]	17 (45)	20 (29)	1.98 (0.87 – 4.52)	0.10	3.09 (1.18 – 8.04)	0.02
PER3[f]	6 (16)	2 (3)	6.28 (1.20 – 32.85)	0.03	11.55 (1.74 – 76.69)	0.01
SFRP4[e]	5 (29)	12 (17)	0.72 (0.23 – 2.23)	0.57	1.09 (0.32 – 3.75)	0.89
SFRP5[b]	5 (29)	5 (7)	1.94 (0.52 – 7.18)	0.32	3.73 (0.8 – 17.14)	0.09
TIMP3[g]	25 (66)	42 (61)	1.24 (0.54 – 2.83)	0.61	1.15 (0.44 – 2.98)	0.78
TMEFF2[h]	21 (55)	33 (48)	1.35 (0.61 – 2.98)	0.46	1.48 (0.64 – 3.42)	0.36
WIF1[i]	15 (39)	24 (35)	1.22 (0.54 – 2.77)	0.63	1.31 (0.53 – 3.24)	0.56

Abbreviations: OR, odds ratio; CI, confidence intervals; a – Adjusted for: vitamin D and multivitamin use; b – Adjusted for: vitamin C and vitamin D use and physical activity; c – Adjusted for: multivitamin , vitamin C, and vitamin D use, physical activity, smoking history, currently drinking alcohol, being married, and age group; d – Adjusted for: vitamin C and D use and ever smoking history; e – OR adjusted for vitamin C and D use; f – Adjusted for: multivitamin and vitamin D use, physical activity, smoking history, and being married; g – Adjusted for: multivitamin and vitamin D use, physical activity, and age group; h – Adjusted for: vitamin D use

Discussion

- **Novel association**: cases were more likely to be hypomethylated in the promoters of the *MINT1*, *PER1*, and *PER3* genes in PBLs compared to controls
  - Thought to function as tumor suppressors
    - Methylation of non-target tissue (i.e., blood) has been shown to be influenced by the environment, dietary factors, and current health status
    - May have only detected a phase shift of methylation
- Need for future larger studies

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