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DNA/RNA markers for colorectal cancer risk in preserved stool specimens: a pilot study

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ABSTRACT

Aims and background. Exfoliated cells in human stool offer excellent opportunities to non-invasively detect molecular markers associated with colorectal tumorigenesis, and to evaluate the effects of exposures to exogenous and endogenous carcinogenic or chemopreventive substances. This pilot study investigated the feasibility of determining DNA methylation and RNA expression simultaneously in stool specimens treated with a single type of nucleic acid preservatives.

Methods. Stool specimens from 56 volunteers that were preserved up to a week with RNAlater were used in this study. Bisulfite sequencing was used to determine methylation at 27 CpG loci on the estrogen receptor 1 (*ESR1*) promoter. Taqman assay was used for quantitative reverse transcription polymerase chain reactions to measure cyclooxygenase 2 (*COX2*) and epidermal growth factor receptor (*EGFR*) mRNA expression. Subjects' basic demographic and other selected risk factors for colorectal cancer were captured through questionnaires and correlated with the levels of these markers.

Results. Less than 10% of the samples failed in individual assays. Overall, 24.0% of the CpG loci on the *ESR1* promoter were methylated. *COX2* expression and alcohol use were positively correlated; an inverse association was present between *EGFR* expression and cigarette smoking; and subjects using anti-diabetic medication had higher *ESR1* methylation. In addition, higher *EGFR* expression levels were marginally associated with history of polyps and family history of colorectal cancer.

Conclusions. The present study demonstrates that simultaneous analyses for DNA and RNA markers are feasible in stool samples treated with a single type of nucleotide preservatives. Among several associations observed, the association between *EGFR* expression and polyps deserves further investigation as a potential target for colorectal cancer screening. Larger studies are warranted to confirm some of our observations.

Key words: stool test, DNA, RNA, colorectal cancer.

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