Rectal MicroRNA as a Screening Test for Colon Neoplasia: A Pilot Study

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Introduction: Colorectal cancer (CRC) ranks as the second leading cause of cancer deaths in the UK. It is eminently preventable through identification and colonoscopic removal of the precursor lesion, the advanced adenoma (AAs). However, screening is both inefficient and inaccurate. For example, fecal immunohistochemical test (FIT) has a ~25% sensitivity for AAs. On the other hand, colonoscopy has a prevalence of ~6-8% meaning that >90% of screening colonoscopies are squandered with CRC prevention perspective. Finding better risk stratification is critical. Our group has focused on detection of field carcinogenesis, the notion that the genetic/epigenetic milieu that leads to a focal neoplasm can be detected throughout the colon including the readily accessible rectum. This is the clinical rationale for full colonoscopy for polyps on flex sigmoidoscopy or post-polypectomy surveillance ("condemned mucosa"). MicroRNAs (miRs) are small (18-25 nucleotide) molecules that can impact specific gene expression. There are ~3000 species and miRs are resistant to degradation so ideal for detection in the colon.

Methods: Human Subjects: After IRB approved informed consent, we recruited patients undergoing colonoscopy and obtained 6 biopsies of the endoscopically normal rectal mucosa. Of the ~150 subjects we identified 9 with advanced adenomas elsewhere in the colon (cases) and selected 15 matched (negative colonoscopies) controls.

RNA Seq: RNeasy mini kits (Ambion) for mRNA sequencing. Sequencing was performed using the Illumina® NovaSeqTM platform. Differential miR was analyzed. We assessed performance via the area under the receiver operator characteristic curve (AUROC).

Results: We identified 2268 miRNA. Of those 165 miRNA values were statistically significant ($p<0.05$), were >1.2 fold or <0,8 dysregulated. There were 46 microRNAs downregulated and 119 upregulated. The top three miRs were hsa-miR-181b-5p with hsa-let-7d-3p and hsa-miR-4661-5p. The performance is in the table below:

	AUROC
miR-181b-5p	0.81
miR-181b-5p + hsa-let-7d-3p	0.92
miR-181b-5p+ hsa-let-7d-3p+ hsa-miR-4661-5p	0.92

We did leave one out validation to determine 95% confidence interval. For the single hsa-miR-181b-5p and with addition of hsa-let-7d-3p it was [0.76, 0.86] and [0.88, 0.95] respectively.

Conclusions: We show for the first time that a panel of 2 rectal miRs had an excellent performance for diagnosis. It is striking that this simple panel outperformed classic tests such as FIT or

Cologuard (~25% and ~45% respectively). This proof of concept study suggests a simple rectal swab may be able to personalize CRC screening.