Fecal Bacterial Markers for Non-Invasive Diagnosis of Colorectal Cancer

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Technology description

We are inviting expressions of interest (EoI) for commercializing “Fecal Bacterial Markers for Non-invasive Diagnosis of Colorectal Cancer” technology. The innovation is developed by Professor YU Jun, Professor of Department of Medicine & Therapeutics of The Chinese University of Hong Kong

The Technology

This invention describes a probe-based internal control assay for quantificaiton of bacterial DNA content and further duplex qPCR assays for quantification of our newly identified fecal bacterial markers by metagenome sequencing.

The internal control assay is well established and optimized with the following aspects: 1) a degenerate primer-probe set was designed with amplicon size suitable for qPCR quantification (<150 bp) targeting a coserved region of 16S rRNA genes, covering >90% of the eubacterial population within the Ribosomal Database Project Release version 10.8; 2) Using our well-optimized experiment protocol, Cq values correlated well with Log2 DNA quantities (R2 =0.6466).

Duplex qPCR assays involving the internal control and primer-probe sets specifically targeting our newly identified fecal bacterial markers by metagenome sequencing, including m1704941 (with 99.13% sequence identity to butyryl-CoA dehydrogenase gene from F nucleatum), m1696299 (with 99.78% sequence identity to rpoB gene from P. micra), F nucleatum (Fn), B clarus (Bc), Roseburia intestinalis (Ri), Clostridium hathewayi (Ch), and one undefined species (labeled as m7), have been established. Strong positive correlations were demonstrated between the quantification of each bacterial candidate by qPCR assays and metagenomics approach ($r=0.816\sim0.950$, all $P<0.0001$). Using our duplex-qPCR assays, the abundances of these markers have been detected quantitatively in fecal samples from colorectal cancer patients and healthy control subjects, all showing significant enrichments or decreases in colorectal cancer (CRC) patient microbiomes consistently with metagenome sequencing findings. Combined qPCR measurements of m1704941 and m1696299 clearly separated CRC from healthy controls and accurately classified CRC samples with an AUROC of 0.84 (sensitivity=72.3%; specificity=92.7%). Fn abundance was predominantly higher in CRC patients compared with healthy controls ($P<0.0001$), giving an AUROC of 0.8675 ($P<0.0001$). At the best cut-off value, Fn could discriminate CRC from controls with a sensitivity of 77.7% and specificity of 79.5%. A simple linear combination of four bacterial markers (Fn, Bc, Ch and m7) showed an improved diagnostic ability compared to Fn alone, giving an AUROC of 0.8860 ($P<0.0001$). We also showed that quantification of

bacterial markers was significantly more sensitive than the widely used fecal immunochemical test (FIT) for the detection of CRC, especially for early stage CRC.
In summary, we have established a reliable platform for convenient translational application of new bacterial markers. The stool-based CRC-associated bacteria identified by our metagenome sequencing study can serve as novel biomarkers for the non-invasive diagnosis of CRC patients.

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