A multiplex promoter hypermethylation DNA test used as biomarker for blood-based colorectal cancer detection. Jean-Pierre Roperch, Floriane Bard, Solène Forbin, Iradj Sobhani (coordinateur). Service de Gastroentérologie, CIC, IFR, CHU Henri Mondor, 94000 Créteil

Background

Colorectal cancer (CRC) remains a major medical problem in the world. Sooner it is detected, better are chances to cure it. Fecal occult blood test (FOBT) is a current noninvasive screening method for CRC detection. However, it detects less than 50% asymptomatic cancers and lacks of specificity. In this context, new specific CRC biomarkers for diagnosis of early stage tumours are needed. Aberrant DNA hypermethylation is recognized as playing a crucial role during CRC development. The main goal of the study was to develop and validate a CRC-specific methylated DNA test in serum.

Methods

Initially, the GoldenGate Methylation Cancer Panel I containing 1,505 CpG loci within 807 cancer-related genes was used to study methylation patterns in CRC patients. DNA methylation microarray-based assays was performed on 32 tissues (16 CRC and 16 normal) and effluents (20 blood and 20 stool samples). Each effluent includes 10 patients with cancer and 10 healthy subjects as assessed by colonoscopy and pathology findings. Three candidate genes referred to the highest level of methylation were included in a relative quantitative multiplex methylation-specific PCR (QM-MSP). These three genes were evaluated on a additional series of 30 tissues samples (15 CRC and 15 homologous normal tissues). Finally, this test was validated in 224 serum samples (32 CRC and 192 normal).

Results

At the optimal threshold value in QM-MSP, three candidate genes (MK1, MK2, MK3), were tested in DNA serum as either single or combined marker. The sensitivity and specificity for detecting CRC of the combined multiplex hypermethylated DNA test were 78.1% and 89.6% respectively with MK1 showing 59.4% and 90.1% and MK2 56.3% and 89.6% and MK3, 56.3% and 90.1%, respectively as a single gene marker.

Conclusion

Aberrantly methylated DNA multiplex including MK1, MK2 and MK3 is proposed as epigenetic combined biomarkers in a simple blood test for CRC detection.

In progress

1- In screening program schedule including 1000 asymptomatic individuals (50-74 years) (VATNIMAD, PHRC 2009).
2- In symptomatic patients referring for colonoscopy including 500 patients (ANR Biotecs 2010)

Valorisation

• Creation of Profilem S.A.S
• Patented biomarkers
• Article in progress