Detection of Large Rearrangements in Hereditary Colorectal Cancer Using Microarray CGH

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Introduction

Hereditary Colorectal Cancer syndromes include Hereditary Non-polyposis Colorectal Cancer (HNPCC), Familial Adenomatous Polyposis (FAP), and MYH-Associated Polyposis (MAP). Deleterious mutations in the associated genes such as MLH1, MSH2, MSH6 and APC, are amenable to high throughput processing, which increases the efficiency of testing large rearrangements, and contributes to shorter turn-around-times.

Materials and Methods

Microarray CGH provides a quantitative comparison of patient and control DNA binding to multiple probes on a microarray for the detection of large genomic deletions and duplications. A schematic illustration of the process is shown in Figure 1.

Our analysis examined the results from patients who had received full gene sequencing and large rearrangement analysis by microarray CGH. Among mutation-positive patients, we determined that of all the mutations detected, 80/941 (8.5%) of those mutations were large rearrangements in MLH1, MSH2, MSH6, APC, MYH and EPCAM. The probes are tiled to ensure detection of rearrangements that are as small as a few hundred bases in length. To ensure optimal coverage, it was also necessary to place probes in traditionally challenging regions (e.g. GC-rich regions). A stringent selection process was used to create an optimal probe set. The probes designed in silico were tested empirically against known negative and positive samples to produce a microarray CGH assay with enhanced sensitivity and specificity to detect variations in dosage.

Results

Validation consisted of a blinded analysis of 357 DNA samples, of which 286 were derived from blood and 93 from buccal samples. We correctly identified all 88 positives among 327 samples that were previously examined. Sensitivity and specificity were 100%.

Discussion

We have shown that microarray CGH can reliably detect the presence of genomic deletions and duplications in MLH1, MSH2, MSH6, EPCAM, APC, and MYH. With the introduction of microarray CGH technology, we are able to provide higher resolution in the detection of large rearrangements in these genes due to greater probe number and more comprehensive coverage. An additional advantage of the microarray CGH technology is lower susceptibility to technical artifacts caused by single nucleotide polymorphisms at probe binding sites.

As we continue to study the genetic etiology and clinical presentation of Hereditary Colorectal Cancer, we are beginning to appreciate that the phenotypic distinctions between the CRC-related syndromes (HNPCC, FAP and MAP) are less well defined than previously thought. As such, it is becoming increasingly clear that there are advantages in shifting from syndrome testing and toward a more comprehensive analysis of the multiple genes involved in HCC. Use of testing strategies that are strictly syndrome-based has led to potential lost opportunities for mutation detection. For instance, patients with MYH-associated polyposis (MAP) have largely presented with polyposis, which has traditionally been one of the key indicators for genetic testing of the MYH gene. It has recently been demonstrated, however, that a subset of MAP patients present with CRC in the absence of recognizable adenomatous polyposis. In a study conducted by Myriad Genetic Laboratories, it was shown that among 921 patients diagnosed with colon cancer at ≤50 years and with < 10 reported colorectal adenomas, 2.1% carried biallelic MYH mutations. Additionally, it had been common practice to first test for the two MYH founder mutations, Y165C and G382D, followed by full MYH sequencing only if one of the founder mutations was detected. However, a retrospective analysis by Myriad Genetic Laboratories showed that of 1295 patients who underwent full MYH sequencing, 85 were biallelic for an MYH mutation. Of these 85 individuals, 21 (or 25%) carried two non-founder mutations. Finally, a significant proportion of MAP patients are found to be monoallelic carriers of an MYH sequencing mutation, which has uncertain clinical implications. It is possible that some of these patients also carry a large rearrangement in MYH. At present, we have detected two large rearrangement mutations in MYH, a full deletion of exon 7 and a whole gene duplication. Therefore, with the introduction of clinical testing for MYH large rearrangements by microarray CGH, we are poised to increase the clinical sensitivity of the test, expand the mutational spectrum associated with MAP and potentially identify additional biallelic MYH mutation carriers.

In all, microarray CGH is a clinically validated test that accurately detects deletions and duplications for multiple genes involved in Hereditary Colorectal Cancer. Large rearrangements constitute a significant proportion of mutations found in patients with hereditary CRC, and is part of a comprehensive genetic testing strategy.

References