

Abstract Information

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Sample Abstract

Quantitative Analysis of Microsatellite Instability. M.I. Coolbaugh-Murphy¹, P. Papageorgiou¹, M. Frazier², D.G. Monckton³, M. J. Siciliano¹.

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Microsatellite instability has been demonstrated in a variety of tumor types and is associated with several DNA repair gene mutations. However, current techniques do not have the sensitivity to quantitatively analyze the mutation frequency at an individual microsatellite locus. Such data would be necessary to assess bias for mutations at loci representing different repeat conformations where instability is caused by mutations at different repair gene loci. This has been shown in yeast where repeats of different unit and array size demonstrated variable sensitivity to mutations in different repair genes. Mutation frequency across a panel of microsatellite loci could be used to construct a mutational "signature" for various tumor types and/or repair gene mutations. An established mutational signature could be used in instances where a specific gene defect has not been characterized, to help identify new tumor suppressor genes, to detect minimal residual disease, or to identify at-risk individuals presymptomatically. By combining the quantitative power of Small-Pool PCR (SP-PCR) with high-throughput fluorescent multiplex PCR analysis, it was our aim to be able to examine tumor material in a manner to respond to these questions. Here we use the trinucleotide microsatellite at the DM locus to demonstrate this approach to be feasible and capable of achieving the objectives. HNPCC tumor material with an identified repair gene defect (MLHI) was used to prove this principle. While resolution of microsatellite alleles via radioactively end-labeled primer PCR separated on acrylamide gels was routine, SP-PCR's resulted in separation of individual alleles of a heterozygote into individual PCR reactions at 1 genome equivalent (g.e.) of input DNA. This enables the detection of small, low frequency repeat mutations not seen at higher DNA input levels. Fluorescent analysis of a "reconstruction" experiment detects 1/1000 mutations when multiple 100 g.e. pools are screened. This particular tumor turned out to have a mutation frequency at the DM locus of 0.03, greater than the background level of <1/1,000. (p> 0.001) Standard analysis of this material, using 4,000-40,000 g.e., would not have revealed the instability at this

locus, nor would it have been a quantifiable result, in terms of mutation frequency. Finally, conditions for the fluorescent detection of individuals alleles of a heterozygote separated into 1g.e. have been defined. Therefore a sensitive, quantitative technique that can be applied in a high throughput manner to allow analysis of mutational spectra at microsatellite loci has been developed. This technology can now be applied to address the genetic and biological basis of microsatellite instability.

Presenter:	Mary		Coolbaugh-Murphy
Presentation	category:	Post	doctoral
Consideration for award:	yes		