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Use of genotypic selection to detect *P53* codon 273 CGT > CTT transversion: Application to an occupationally exposed population

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Abstract

CGT > CTT transversion in codon 273 of the *P53* tumor-suppressor gene is one of the major mutations detected in human tumors. Within an epidemiological framework, we investigated the use of a genotypic selection method to measure this point mutation. The allele-specific polymerase chain reaction (AS-PCR) that was developed was able to detect 10 mutant copies of the gene among a total of 5×10^5 wild-type copies. We used this assay to detect CGT > CTT transversions in buccal cell DNA of production workers ($n = 76$) from a viscose factory exposed to carbon disulfide (amongst other pollutants) and in the DNA of non-exposed office workers ($n = 67$). The mutation appeared more frequently in the exposed than in the non-exposed worker who were smokers. The results of the study indicate that occupational exposure results in a significant increase in *P53* CGT > CTT transversions and more especially identified occupational exposure in combination with smoking as a significant risk factor for the mutation. We conclude that AS-PCR of the *P53* 273rd codon transversions is a suitable technique for studying the effects of occupational exposure.

Keywords: AS-PCR; Point mutation; Molecular epidemiology; Occupational exposure

Introduction

Over the last 30 years, the introduction of biomarkers has opened new avenues for conducting epidemiological studies. In this regard, progress in molecular biology has provided new tools and enabled a better understanding of links existing between exposure and pathology (Boffetta, 2000).

In epidemiology studies of cancer, it is often difficult to connect pathology with exposure because of both the relatively low incidence rates of disease and the long latency period between exposure and disease. For these reasons, it is an attractive option to relate exposure to an intermediate biomarker rather than to the final disease (Bartsch, 2000; Furberg and Ambrosone, 2001). Investigators have attempted to use intermediate lesions, in particular lesions at the DNA level, for this purpose (Vainio, 1998). To do so, it is of paramount importance that the sensitivity of the assay is sufficient to detect the intermediate marker. Different methods have been developed to detect these intermediate lesions; initial studies commonly used cytogenetic analysis, with

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molecular analysis becoming more common in recent years (D'Surney et al., 2001; Mashal and Sklar, 1996).

Numerous papers have described the relevance for cancer of point mutation in oncogenes and tumor-suppressor genes such as *P53*, *KRAS* or *RB* (Behn et al., 1998; Bennett et al., 1999; Burnouf et al., 2000; Sidransky, 1997). Several studies have used genotypic selection methods coupled to polymerase chain reaction (PCR) to detect point mutations in these genes (McKinzie et al., 2001). Some of these methods are based on polymerase fidelity during the amplification step; the polymerase inefficiently elongates a primer when there is a mismatch between the DNA of the target sequence and the 3′ end of the primer. In these assays, the primer is designed to hybridize to the mutant allele but not to the wild-type allele. Ideally, only mutated template copies are amplified; however, some mismatches template extensions by the polymerase result in amplification of the wild type. The nature of the mismatch plays an important role in the hybridization and the specificity by which the PCR primer is elongated (Kwok et al., 1994).

In order to increase the specificity of the amplification, investigators have manipulated the number and type of mismatches. Depending on the sensitivity of the assay, genotypic selection can be used for different purposes, like mutation detection or genotyping. Indeed, the frequency of spontaneous mutation in biological systems can be near 10^{-8}/base pair, depending on the sequence measured and the methods used to detect mutant frequency. However, hot spots are known to exist that have a higher spontaneous mutation frequency. For example, the overall spontaneous mutation frequency for the rat genome is 5×10^{-9}, but it is close to 5×10^{-6} for the *Hprt* locus (Cole and Skopek, 1994). If we consider the impact of a genotoxicant, we may expect an increase of 10- to 100-fold, and the hot spot mutation frequency may be in the order of 5×10^{-4} to 5×10^{-5} (McKinzie et al., 2001; Parsons and Heflich, 1997). Measuring induced mutation frequencies require much more sensitive methods than those used for genotyping, where there is a need to distinguish only three different frequencies: 1 (homo-), 0.5 (hetero-) and 0 (nulli-zygosity). The kind of frequencies is nearly the same when analyzing point mutations on tumorous cells and the genotypic selection methods are generally used in this way.

In its basic form, allele-specific-PCR (AS-PCR) is a technique that uses a primer with a single-nucleotide mismatch at its 3′-end when hybridized to the wild-type target allele and no mismatch when hybridized to the mutant allele. AS-PCR is currently the most frequently used genotypic selection technique, and can be used for the detection of point mutations in non-neoplastic tissues (Kirby et al., 1996) and cancer (Hodgson et al., 2001; Tada et al., 1993), the analysis of single nucleotide polymorphism in genes (Chen et al., 2001), and even the classification of micro-organisms (Akada et al., 2001). The mismatch amplification mutation assay-PCR (MAMA-PCR) is a modification of AS-PCR that uses a primer having two mismatches to the wild-type allele and one to the mutant allele. The addition of one mismatch decreases non-specific amplification of the wild-type allele (related to the error frequency of the polymerase), and thus increases the sensitivity of the assay. When applied to detecting mutation in the 12th codon of the *KRAS* gene, MAMA-PCR is capable of detecting 30 mutated copies amongst three million wild-type copies (Cha et al., 1994, 1992). Additional modifications, like Taq-MAMA or ARMS, use real-time PCR primers (Clayton et al., 2000; Glaab and Skopek, 1999). Finally, ACB-PCR (allele-specific competitive blocker-PCR) includes a non-extendable primer with one mismatch to the mutant allele and no mismatch to the wild-type allele (McKinzie and Parsons, 2002). This non-extendable primer preferentially blocks the amplification of the wild-type allele when the addition of a second mismatch on the extendable primer inefficiently decreases amplification of the wild-type allele.

In the present study, we have evaluated the usefulness of genotypic selection for detecting the genotoxicity resulting from occupational exposure. The aim of this study was to determine, within an epidemiological framework, the potential of these techniques for detecting the consequences of exposure. In this study, we have measured mutation in the human *P53* gene, because of its great significance to carcinogenesis (Soussi, 1996, 2003). There is little information available on the application of AS-PCR for detecting induced mutation in some hot spots of the *P53* gene; for this study we have chosen G > T transversion of the second base of *P53* codon 273. A high frequency of *P53* CGT > CTT mutation is a distinctive feature of lung tumors from patients with smoking history (Olivier et al., 2002) and is commonly believed to result from the direct or indirect action of exogenous compounds like polycyclic aromatic hydrocarbons (Pfeifer and Hainaut, 2003; Rodin and Rodin, 2004). There has been only one study measuring CGT > TGT transition in *P53* codon 273 using AS-PCR (Rhodes et al., 1997). Compared to the transitions, transversion in this codon appears more relevant for the study of environmental genotoxic compounds.

Our study addresses the following questions:

- Which is the better AS-PCR primer design for detecting CGT > CTT transversion, a primer containing one or two mismatches?
- What sensitivity can be reached using this technique? In order to analyze the sensitivity of the technique, mixtures of mutant and wild-type genomic DNA