Common DYPD Mutation Associated with 5-Fluorouracil Toxicity Detected by PCR-mediated Site-directed Mutagenesis

To the Editor:

The human dihydropyrimidine dehydrogenase gene (DYPD) encodes dihydropyrimidine dehydrogenase (DPD; EC 1.3.1.2), the first and rate-limiting enzyme in the three-step pathway of uracil and thymine catabolism. DPD is also the principal enzyme involved in detoxification of pyrimidine-based antimetabolic analogs, such as 5-fluorouracil (5-FU), a drug that is commonly used in the treatment of solid tumors (colon, breast, head, neck, ovary, and skin). Because >80% of the administered 5-FU is degraded by DPD (1), the DPD catalytic activity in cancer patients could affect the efficacy of 5-FU treatment. In cancer patients with very low DPD activity, toxic reactions (e.g., diarrhea, stomatitis, mucositis, myelosuppression, and neurotoxicity) were reported that in some cases were life-threatening and sometimes fatal (2). A frequency as high as 3% of putative heterozygotes for DPD deficiency was also estimated based on catalytic activities in population studies (3, 4). The identification and characterization of the human DPD cDNA (5) made possible the identification and molecular analysis of mutations that affect DPD expression and catalytic activity. The most common mutation (6) associated with severe toxicity is a G→A transition at the 5′-splicing donor consensus sequence in intron 14 that leads to exon 14 skipping (7–10): c.1905+1G→A [according to mutation nomenclature (11)]. By itself, the G-to-A nucleotide change destroys a unique restriction site only for the expensive MaeII endonuclease (isoschizomers, Tail and Tscl). We used PCR-mediated site-directed mutagenesis (PSM) utilizing a PCR primer with a single-base mismatch near the mutation site to introduce into the amplified wild-type product an allele-specific SnaBI restriction site. In this way, the amplification product encompassing this polymorphic site can be restriction-digested and electrophoresed to resolve alleles easily.

Our strategy was such that the enzyme we used cut the amplified wild type once, but not the amplified homozygous mutant type. This choice provides in most cases a positive control for the SnaBI digestion. Using the Hardy-Weinberg equilibrium, the frequency of heterozygotes allows the estimation of up to 1 in 1000 homozygotes for DYPD mutations. We designed in the 3′ end of exon 14 of the DYPD gene the forward primer DPD-PSM (5′-CTAAAGGCTGACTTCCAGAC-TAC-3′) to contain a single-base mismatch (A→T), creating a novel SnaBI restriction site (TAC↓GTA) in the amplified wild-type allele. DPD-PSM was designed based on the sequence from GenBank accession no. U20938. The reverse primer DPD-delR (5′-CAGCAAAAGCAACTG-GCAGATTCC-3′) was located in intron 14 (10).

The amplification product length before digestion was 155 bp. Digestion by SnaBI restriction endonuclease generated two fragments (131 and 24 bp; the 24-bp fragment migrates quickly and is not seen on the gel) in the wild-type allele (Fig. 1) and does not cut the product from the homozygous mutant c.1905+1G→A allele (not shown). After electrophoresis in an agarose gel, a heterozygote for the mutation theoretically shows three bands of 155, 131, and 24 bp, which correspond to the two alleles (Fig. 1).

Because 5-FU is one of the most commonly prescribed chemotherapeutic drugs in cancer treatment (in monotherapy or polytherapy) and the c.1905+1G→A mutation is frequently linked to severe toxicity, molecular screening of cancer patients could be done routinely, coupled with analysis of DPD activity in peripheral blood mononuclear cells, before the start of treatment to avoid the toxic effects of 5-FU. Because economic problems are very important in health-screening strategies, screening tests must be the least expensive. The use of SnaBI in a PSM method produces an 18-fold decrease in the enzyme cost ($0.70 vs $12.87 US per reaction) compared with the previous PCR-restriction method using MaeII (7, 8).

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References


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