Novel Thymidylate Synthase Germline Polymorphism Predicting Increased Toxicity Risk

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ABSTRACT

INTRODUCTION: 5-Fluorouracil (5-FU) chemotherapy in combination with leucovorin is the standard treatment for advanced colorectal adenocarcinoma. 5-FU targets the enzyme thymidylate synthase (TS), an anabolic enzyme involved in DNA synthesis, especially in rapidly dividing cancer cells. This provides the only de novo source of thymydilate for DNA synthesis. Contradictory data have been reported in the literature correlating germline polymorphisms in the 5’-promoter region (5’-TSER) and the 3’-untranslated region (3’-UTR) of the TYMS gene to mRNA expression levels, responsiveness to 5-FU therapy, and clinical outcome. We have detected a novel 5’-TSER polymorphism, 4RGC, which predicts low mRNA expression based on diminished transcription factor binding sites, and an increased risk of 5-FU toxicity.

METHODS: The evaluation of polymorphisms included a 6 base pair deletion (rs16430) of the 3’-UTR (DEL) versus the wild type insertion (INS), and a variable number (either 2 or 3) of tandem repeats (2R, 3R) of the 5’-TSER (rs1032210), as well as a G>C SNP (rs2535424) in the second repeat of the 3R allele (3RGGC, 3RGCC). The 5’-TSER and 3’-UTR enzyme areas were amplified by PCR. In addition, a portion of the 5’-TSER amplification was digested with the restriction enzyme Hae III to discern the G>C SNP. The first Hae III cut site within the 5’-TSER region of the 2R allele (2RGC) is mutated to create an amplicon with a gGc sequence instead of gGc cut site. This design eliminates the creation of an uninformative 11 bp product. Instead, the 2RG and 3RGGC amplicons will both yield a labeled digest product of 77 bp, and the 3RGGC amplicon will yield a labeled digest product of 105 bp (Figure 1).

RESULTS: The patient’s 3’-UTR TYMS genotype is DEL/DEL homozygous for the 3’-UTR DEL (rs16430) polymorphism. The patient’s 5’-TSER TYMS genotype is 4RGC/3RGCC. Initial NGS studies support the PCR and Sanger sequencing results. This previously unreported 4RGC/3RGCC polymorphism has only one functional transcription factor binding site, and is therefore predicted to have lower mRNA expression similar to a 2R allele.

CONCLUSIONS: In routine clinical testing, novel, rare variants can be identified. Although functional predictions may be made, the demonstration that communication between the clinical laboratory and treating clinicians, and correlation with the clinical scenario are needed to increase our ability to guide treatment and provide prognostic information. Germline polymorphism screening of the TYMS gene continues to evolve.

MATERIALS & METHODS

5-FU is the most commonly used fluoropyrimidine for the routine treatment of many types of cancer including colorectal cancer. When 5-FU is administered, approximately 60 percent is catabolized by the enzyme dihydropyrimidine dehydrogenase (DPD). The remaining 40 percent is converted to toxic metabolites in the cytosol. The remaining drug is further metabolized into an active form that inhibits the synthesis of both DNA and RNA by either competitive inhibition of TS enzyme or direct incorporation into DNA as toxic pyrimidine analogues. The TS enzyme provides the only means for the synthesis of both DNA and RNA by either competitive inhibition of TS enzyme or direct incorporation into DNA as toxic pyrimidine analogues. The TS enzyme provides the only means for the synthesis of DNA synthesis, and accordingly, its expression is higher in rapidly proliferating cells. Therefore, we developed a genotyping assay to assess germline polymorphisms in the 3’-UTR and 5’-TSER of the TYMS gene.

5’-TSER genotypes 2RGC/3RGGC, 3RGGC/3RGCC and 4RGC/3RGGC are considered high expression genotypes often associated with poor 5-FU responsiveness (G indicates a functional E-box according to Lincz et al.). In contrast, genotypes of 2RGC/2RGC, 2RGC/3RGCC, and 3RGGC/3RGC are considered low expression genotypes associated with good 5-FU responsiveness, and in some cases, increased risk of 5-FU toxicity. Therefore, we developed a genotyping assay to assess germline polymorphisms in the 3’-UTR and 5’-TSER of the TYMS gene. A novel mutation was identified and is the subject of this report.

PCR and restriction enzyme digest products were then combined and detected by capillary electrophoresis on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Data was analyzed with GeneMarker software (Soft Genetics). Sanger Sequencing: DNA was amplified with the following primers (Soft Genetics):

- Forward Primer: 5’-CGAGGCTGTGAACGAGAACAG-3’
- Reverse Primer: 5’-CTCCGAGCCGGCACAGGCATGGCGCGGCG-3’

This design eliminates the creation of an uninformative 11 bp product. Instead, the 2RGC and 3RGCC amplicons will both yield a labeled digest product of 77 bp, and the 3RGGC amplicon will yield a labeled digest product of 105 bp (Figure 1).

Next Generation Sequencing: PCR enrichment of the TYMS 5’-TSER area was accomplished using the following forward and reverse primers: 5’-TGCGCCCTTGCGTTCCCCCT-3’ and 5’-ATGCGGAAATTCCGAGCCGGTTTTCTGTTGTCCG-3’. Libraries were prepared using the Nextera® XT DNA Sample Preparation Kit (Illumina), following a modified protocol, which excludes the bar-code normalization step. Samples were sequenced on the Illumina MiSeq using the 600 cycle (300x300), version 3 reagent kit.

RESULTS: The 3’-UTR 6 bp deletion has been shown to decrease TYMS mRNA stability, leading to reduced TS expression. The 28 bp tandem repeats contain an enhancer box (E-box) site necessary for the transcription of the thymidylate synthase gene. The 3’-UTR 6 bp deletion has been shown to decrease TS expression by up to 70%.

Conclusions: We have identified a novel 5’-TSER polymorphism, 4RGC, following the nomenclature proposed by Lincz et al. The patient’s TYMS genotype, 3RGCC/4RGC, which is based on the number of E-box sites, would predict lower TYMS mRNA expression similar to 2RGC, and thus better 5-FU response and increased risk of toxicity.

In routine clinical testing, novel, rare variants can be identified. Although functional predictions can be made, this case is another demonstration that communication between the clinical laboratory and treating clinicians, and correlation with the clinical scenario is needed to increase our ability to guide treatment and provide prognostic information. Germline polymorphism screening of the TYMS gene continues to evolve.

REFERENCES


FIGURE 3: Sanger sequencing electropherogram.