The Role of Thymidylate Synthase as a Molecular Biomarker

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Significant advances have been made in the treatment of advanced colorectal cancer. At present, there are four main active drugs to treat the disease, and they include the fluoropyrimidines, 5-fluorouracil (5-FU) and capecitabine, irinotecan, and oxaliplatin. Over the past few years, intense research efforts have focused on developing molecular biomarkers that could predict response to these various agents. There are now a number of clinical studies demonstrating that patients with metastatic colorectal cancer and with tumors that express high levels of thymidylate synthase (TS) have a decreased response to 5-FU-based chemotherapy (reviewed in Ref. 1). Several methods have been developed to measure TS expression, and they include immunohistochemistry for protein levels, reverse transcription-PCR for RNA levels, and determination of genetic polymorphisms for DNA expression. In particular, the ability to correlate the presence of TS polymorphisms in peripheral blood with response to 5-FU chemotherapy has received great attention of late because this represents a relatively noninvasive approach for molecular profiling.

The TS gene contains a unique 28-bp tandem repeat sequence within the 5\textprime;-untranslated region, and patients with two tandem repeats (2R/2R; TSER\textsuperscript{*2}), three tandem repeats (3R/3R; TSER\textsuperscript{*3}), and/or a heterozygous 2R/3R (TSER\textsuperscript{*2}/TSER\textsuperscript{*3}) genotype have been identified (2, 3). This tandem repeat sequence appears to function as an enhancer element because preclinical in vitro studies have shown that stepwise increases in TS mRNA expression and TS enzyme activity as well as TS expression are associated with increasing number of repeat sequences. This finding has been extended to the clinical setting, where studies have documented that TS mRNA expression was significantly higher in patients homozygous for TSER\textsuperscript{*3} than in those expressing the TSER\textsuperscript{*2} genotype. With regard to its role as a predictive biomarker, there is growing evidence that the TSER genotype is associated with clinical efficacy, in terms of response and survival, to 5-FU-based chemotherapy (3).

These initial observations showing correlations between the presence of TS polymorphisms and tumor response to 5-FU chemotherapy have generated a great deal of interest because the prediction of tumor response could be achieved using easily accessible normal tissue such as peripheral blood. However, a high incidence of loss of heterozygosity at chromosome 18, by as much as 60–65%, has been observed previously at the TS locus in tumor tissues (4, 5). This loss of heterozygosity would then lead to modification of the TS genotype in the tumor tissue, thereby resulting in a different genotype than that present in normal tissue. In this regard, the paper by Uchida et al. (6) presented in this issue of Clinical Cancer Research is of particular relevance. In their cohort of patients, the authors observed a high frequency of loss of heterozygosity (77%) at the TS locus. This frequency is higher than the incidence reported previously; however, the authors used laser capture microscopy to directly isolate tumor cells and were thus able to more completely eliminate contaminating normal and/or stromal tissue. Perhaps of even greater significance, they showed that patients with the heterozygous 2R/3R genotype in their normal tissue segregate into two different TS genotypes in their tumor tissue. Patients with the 2R/2R genotype in their tumor tissue had significantly improved outcome with regard to response rate and survival in response to treatment with the oral fluoropyrimidine prodrug S-1 when compared with those expressing the 3R/3R genotype. This study is important because it demonstrates that genotyping of normal tissue is clearly insufficient as a marker for response to TS-directed therapy and emphasizes the need to determine the TS polymorphism status in tumor tissue.

This study by Uchida et al. (6) provides further support to the growing body of evidence that TS expression may be used as a molecular biomarker of response to TS-directed chemotherapy. However, there are a number of issues that must be addressed before such molecular profiling studies can be brought into standard clinical practice. First, it should be emphasized that the mechanism(s) by which the TSER polymorphism affects tumor response remains to be established. It is clear that for this and any other polymorphism identified, preclinical biochemical and/or molecular studies must be performed to confirm that these polymorphisms result in a biological effect. The absence of a well-defined functional consequence would raise serious doubts as to their actual clinical relevance. Second, the control mechanisms by which TS expression is regulated are quite complex, and there is growing evidence that multiple levels of control are involved, including transcription, posttranscription, translation, and posttranslation (7). Along these lines, one would anticipate that the expression of TS represents a dynamic process that fluctuates over a given period of time, dependent on the cellular environment. As such, it may not be realistic to think that measurement of TS expression at a fixed time period is truly representative of TS levels that are expressed in normal and malignant tissues. Moreover, the particular cellular context and various factors relating to a given cytotoxic stress such as dose and schedule of drug administration must be taken into account. As a result, it remains unclear as to whether a polymorphism in the TS gene can, in fact, lead to alterations in expression of TS protein and/or enzyme activity. Third, whereas focus has been placed on the TS polymorphism, it would not be surprising to identify other polymorphisms in genes encoding proteins involved in 5-FU metabolism. For example, the expression of

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dihydropyrimidine dehydrogenase, the key enzyme involved in the catabolic breakdown of 5-FU, dUTP nucleotidohydrolase, the enzyme involved in repairing DNA, and mismatch repair enzymes have all been shown to play a role in determining response to 5-FU chemotherapy. In addition, other enzymes involved in the formation of cytotoxic 5-FU metabolites have been implicated as key response determinants. There is growing evidence that inhibition of TS, by itself, is insufficient for cell death but rather initiates a series of intracellular signaling events that involve cell cycle checkpoint control and apoptosis, which eventually leads to cell death. As a further level of complexity, studies have shown that TS, in addition to its catalytic functions, serves as an RNA-binding protein (8). In this regard, it functions as a translational regulator of genes that may play a critical role in cell cycle control and apoptosis, including p53 and the myc family of transcription factors. Thus, it will be critically important to develop a more comprehensive molecular profile before one can make definitive predictions as to which biomarker accurately predicts for drug response. Finally, there are technical issues that must be resolved. Perhaps of greatest importance is the urgent need to standardize the method for TS genotyping and then validate this method with other currently available methods for determining TS expression, including reverse transcription-PCR and immunohistochemistry.

Of all of the molecular biomarkers identified to date, TS has been the most extensively investigated as a predictive marker for tumor response to TS-targeted agents. However, additional clinical studies are required to firmly establish the relationship between TSER status and clinical response. The rapid advances in biotechnology, including the development of tissue microarrays and advanced bioinformatics systems, will help guide the availability of rapid, inexpensive, valid, and reliable assay systems for the molecular profiling of patient’s tumor. These advances will enable the clinician to shift from administering chemotherapy on a purely empiric basis to a situation in which tailored therapy can be offered to individual patients.

References