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Thymidylate synthase as an oncogene?

A surprising finding in the report by Rahman et al. in this issue of *Cancer Cell* is that forced overexpression of human thymidylate synthase transforms immortalized murine cells into a malignant phenotype. We discuss the possibility that elevated levels of thymidylate synthase noted in some human malignancies may contribute to tumor progression and may also reflect increased levels of its transcriptional activator E2F-1.

In this issue of *Cancer Cell*, Rahman et al. present data showing that high levels of thymidylate synthase (TS), generated by the transduction of immortalized mouse cell lines with human TS cDNA or by induction via a tetracycline-responsive promoter, result in transformation. By the criteria of anchorage independence and tumor formation in nude mice, they show that a high level of TS is capable of transforming these cells to malignancy. Thus, TS may be considered a protooncogene in that it is a normal protein that functions as a positive regulator of growth and is converted to an oncogene by overexpression.

Does a high level of TS expression contribute to tumor growth in patients? Studies show that most metastatic colorectal cancers have high levels of TS mRNA and protein as compared to primary colorectal cancers and that primary colorectal cancers have higher levels of TS as compared to normal colonic tissue. Several studies also show that patients with metastatic colorectal cancer with tumors that have high levels of TS are less likely to respond to treatment with 5-fluorouracil than patients with lower levels of this enzyme (reviewed by Aschele et al., 2002; Bertino and Banerjee, 2003; vanTriest and Peters, 1999). Only 20%–30% of patients have what are considered low levels of this enzyme, possibly related to deletion of a portion of one of the alleles of chromosome 18, the location of the TS gene (Bardot et al., 1991). Of interest, high TS levels in the primary tumor have been associated with a poor outcome, regardless of treatment (Edler et al., 2002).

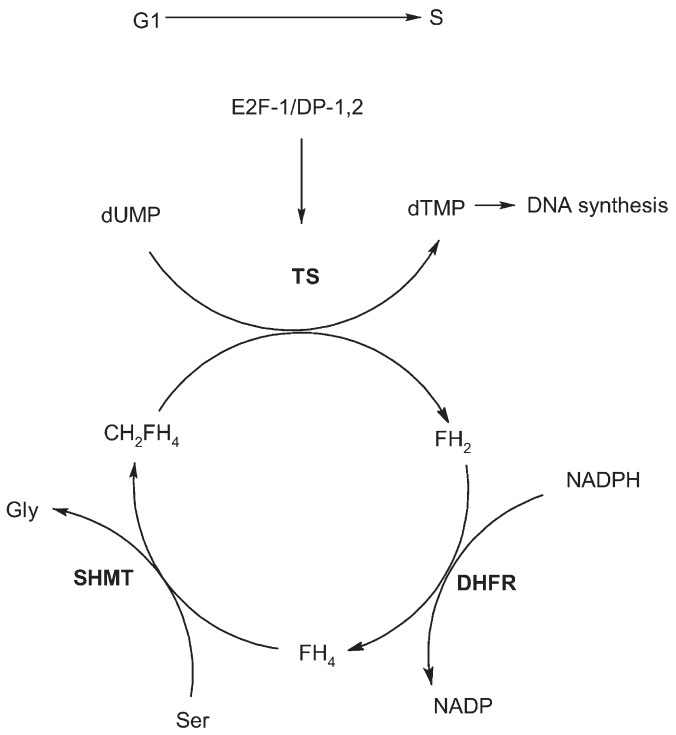

Figure 1. The thymidylate cycle

E2F-1 as the heterodimer E2F-1/DP-1 activates transcription of TS. The other two enzymes of the thymidylate cycle are dihydrofolate reductase (DHFR) and serine hydroxymethyltransferase (SHMT). FH_2, dihydrofolate; FH_4, tetrahydrofolate; CH_2FH_4, 5-10-methylene tetrahydrofolate.

The high levels of TS in some tumors have been attributed to one of two mechanisms: an increase in gene copies of TS (only 5%–7%) or increased transcriptional activation as a result of the transcription factor E2F-1 (Degregori et al., 1995). E2F-1 heterodimerizes with DP-1 and DP-2 and activates the transcription of genes encoding DNA synthetic enzymes, including TS (Banerjee et al., 1998). Interestingly, E2F-1 has itself been described as an oncogene or an oncogenic messenger. Indeed, a recent report indicates that most metastatic colorectal tumors overexpress E2F-1, associated with amplification of this gene, raising the possibility that TS is simply a player in malignant transformation attributed to E2F-1 (Iwamoto et al., 2004).

A second question that arises is whether or not the oncogenic activity that is attributed to TS is related to its enzyme activity. TS is a

well-studied enzyme because of its key role in DNA synthesis and, as such, is a target for anticancer drug development. 5-fluorouracil and fluorodeoxyuridine, clinically useful drugs, when converted to 5-fluorodeoxyuridylate is a potent competitive inhibitor of dUMP, the substrate for this enzyme. Competitive inhibitors of the coenzyme for this reaction, 5-10-methylene tetrahydrofolate, have also been developed, and two of these, raltitexed and pemetrexed, have been approved for use in patients with colorectal cancer (in Europe and Canada) and mesothelioma, respectively. TS is the rate- limiting enzyme in the thymidylate cycle; thus, the rate of thymidylate synthesis is controlled by the level of TS and the concentration of its substrate dUMP and its coenzyme 5-10-methylene tetrahydrofolate (see Figure 1). In this regard, it should be noted that activity measured in cell lysates, in which concentrations of dUMP and 5-10-methylenetetrahydrofolate are much higher than Km values, may not accurately reflect TS activity in the cell, where these concentrations are usually less than or equal to Km. Why is this important? The premise of the authors is that it is the increase in enzyme *activity* that drives cells toward malignant transformation. They support this premise by showing that the overexpression of TS proteins that lack enzymatic activity did not cause transformation in NIH-3T3 cells or tumor formation. While this is a plausible hypothesis, an alternate explanation that should be considered is that elevated levels of TS act as a proliferative stimulus through a nonenzymatic mechanism that is also abrogated by the mutations that affect activity. Indeed, TS has been shown to be a promiscuous protein that binds to TS mRNA as well as to several other mRNAs, including those for c-Myc and p53 (Liu et al., 2002). Binding to its cognate mRNA has been shown to inhibit translation. Treatment of cells with 5-fluorouracil results in an increase of TS protein by relieving this translational inhibition (Liu et al., 2002). A recent study by Berger and associates suggests that this increase may also be a result of decreased degradation of this protein when bound to the inhibitor (Forsthoefel et al., 2004).

In summary, an alternative scenario to the one proposed by Rahman et al. (2004) is that TS is upregulated due to the overexpression of the protooncogene E2F-1 and effects an oncogenic phenotype through a nonenzymatic coopting of mRNAs involved in the expression of growth regulatory genes. In this case, TS itself is not acting as an oncogene or even as an enzyme. However, regardless of which strategy for transformation is confirmed, a central role for TS in tumorigenicity seems apparent, and the report in this issue should stimulate additional enquiry into the functions of TS and encourage continued development of methods to interfere with these function(s).

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