**Time to test CLL p53 function**

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Chronic lymphocytic leukemia (CLL) patients with p53 pathway dysfunction have poor responses to conventional chemoimmunotherapy and short survival. This small but important cohort of CLL patients would benefit from early identification and appropriate management. The challenge is to develop clinically useful tests of p53 pathway function.

There has been considerable progress in analyzing p53 pathway integrity in CLL. Routine fluorescent in situ hybridization (FISH) analysis detects deletion of the region of chromosome 17 (17p13—), which includes the gene (TP53) coding for p53. 17p13— is a powerful predictor of resistance to therapy with purine analogues and alkylating agents and poor prognosis in CLL. Approximately 80% of CLL patients with 17p13— have loss of p53 function because of mutation in the remaining TP53 allele. In contrast, a minority of patients with 17p13— have a more indolent clinical course, which suggests that they retain some p53 function. CLL patients with mutant TP53 without 17p13— also have a poor prognosis. However, the biological consequences of monoallelic mutations of TP53 and the different types of mutations in TP53 on p53 function have still to be fully defined.

In addition, defects in other components of the p53 pathway can affect function. Deletion of the ATM gene on chromosome 11 (11q22—) and increased MDM2 activity are known to decrease p53 pathway activity in CLL. Other components of the p53 pathway (miR-34a) that could contribute to the development of a clinically useful assay of p53 pathway function in CLL.

TP53 mutations can be detected by screening assays and defined by direct sequencing of the coding exons 2-11. These analyses will not detect mutations in the noncoding or regulatory regions that affect gene expression and are not informative about other functional defects in the p53 pathway (see figure). p53 pathway function can be assayed by following exposure to ionizing radiation or with yeast culture–based methods that overcome these limitations, but are difficult to perform in routine clinical laboratories. In this issue of Blood, Asslaber et al report the results of a well-designed series of experiments that provide both confirmatory and novel data about the relationship of p53 pathway function, MDM2 single nucleotide polymorphism (SNP)309, and expression of microRNA-34a (miR-34a) that could contribute to the development of a clinically useful assay of p53 pathway function in CLL.

miR-34a is up-regulated by p53 and mediates some of the p53 pathway effects on cell cycling and apoptosis. CLL patients with defective p53 function have decreased miR-34a expression. In addition, decreased levels of miR-34a have been shown to predict poor prognosis and failure to respond to fludarabine-based therapy. Thus, measurement of expression of miR-34a could be used to determine p53 function. However, there are few data on the effect of defects of other components of the p53 pathway on miR-34a expression in CLL.

Asslaber et al provide additional data on the effects of MDM2 and ATM on expression of miR-34a. Because MDM2 accelerates proteolysis of p53, increased expression by the promoter region SNP309 GG genotype, compared with the TT variant, decreases p53 pathway function. In CLL patients, this results in decreased overall and treatment-free survival. Asslaber et al now show that patients with MDM2 SNP309 GG have lower levels of miR-34a. This is both additional evidence that miR-34a expression is a useful measurement of p53 pathway function and supportive of testing inhibitors of MDM2 such as nutlin-3a for treatment of CLL.

These findings by Asslaber et al will need to be confirmed in larger and prospective studies with longer follow-up using response to treatment and overall survival rather than the surrogate end points of treatment-free survival and lymphocyte doubling time. In addition, further studies need to be done to better define the effects on miR-34a expression of mono- versus biallelic TP53 mutations and TP53 deletion with and without mutation in the remaining allele. The sensitivity of the assay, especially for small CLL subclones with p53 pathway dysfunction, will also need to be determined. miR-34a expression has previously been reported to be lower in CLL cells from patients with fludarabine refractory CLL in absence of TP53 mutation/17p13—. Asslaber et al now report that 10% of patients with low levels of miR-34a did not have known defects in the p53 pathway. This suggests that additional defects in the p53 pathway or miR-34a regulation and metabolism could be important in CLL and this will also require further investigation.

Asslaber et al introduce the intriguing possibility that therapeutic interventions could be directed at increasing the levels of miR-34a in CLL. They show that overexpression of miR-34a in CLL cells induced apoptosis. However, this occurred only in cells derived from patients with wild-type p53, and they suggest that this paradoxical result is compatible with previous reports that an intact p53 pathway is required for miR-34a effects. Unfortunately, this result does not offer any new opportunities to develop novel treatment for those patients with p53 pathway dysfunction who are most in need of more effective therapy.
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REFERENCES

Comment on Yeh et al, page 1247

Triple play of H pylori in ITP

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In this issue of Blood, the meticulous study by Yeh and colleagues offers new insight into the mechanism of “immune” TP associated with H pylori infection.

Although association of H pylori infection with a subgroup of ITP is now widely recognized, little is known about the underlying mechanism of the thrombocytopenia (TP). In this issue of Blood, Yeh et al offer new insight on the mechanism of “immune” TP associated with H pylori infection. They document induction of platelet aggregation by H pylori in vitro and show that this effect is strain-dependent. Using both the proaggregatory strain (Hp49503) and nonaggregatory strains (Hp42504, Hp51932), they demonstrate an essential role for P-selectin and Hp IgG antibody (Hp IGs) in H pylori-induced platelet aggregation. This reaction was completely inhibited by anti–P-selectin antibodies. The presence of H pylori was shown by demonstration of Hp–specific urease gene fragment in the aggregates. They propose that binding of bacteria/Hp IGs to platelet FcγRIIA receptor activates platelets to release granules and to induce surface P-selectin and von Willebrand factor, leading to aggregation. They also looked into platelet apoptosis evidenced by annexin V binding and membrane blebbing, and observed that both proaggregatory and nonaggregatory strains induced apoptosis. Taken together, they conclude that platelet aggregation and apoptosis induced by certain strains of H pylori leads to thrombocytopenia.

As we see it, this “triple play” of H pylori is summarized in the figure. First, IgG antibodies to H pylori are generated. Second, H pylori with the IgG antibodies induce platelet activation and movement of P-selectin to the platelet surface. (3) Interaction of H pylori, Hp IGs, and P-selectin leads to platelet aggregation and apoptosis, reducing the number of circulating platelets. Professional illustration by Paulette Dennis.

diopathic thrombocytopenic purpura (ITP) is the most common autoimmune blood disorder, affecting both children and adults. The term “idiopathic” was coined because in the majority of cases, the underlying causes were unknown. In recent years, however, the list of etiologies of ITP has been steadily increasing, so the term “idiopathic” is becoming obsolete, increasingly replaced by “immune” TP. The recent entry of Helicobacter pylori to the growing list of etiologies has stirred much interest. The initial report from Italy,1 that eradication of H pylori improved ITP, was soon seconded by reports from Japan,2 then dampened by negative results from the United States.3,4 It was suggested that the discrepancy might be due to different strains of H pylori in these widely separated continents.

PLATELETS & THROMBOPOIESIS

(1) IgG antibodies to Helicobacter pylori are generated. (2) H pylori with the IgG antibodies induce platelet activation and movement of P-selectin to the platelet surface. (3) Interaction of H pylori, Hp IGs, and P-selectin leads to platelet aggregation and apoptosis, reducing the number of circulating platelets. Professional illustration by Paulette Dennis.

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