Response

5-azacytidine induction of human fetal hemoglobin

We thank Dr Lavelle and his colleagues for their letter. Their comments are related to 2 major conclusions from our paper: that the induction of fetal globin gene expression by 5-azacytidine (5-Aza) is not the result of global DNA hypomethylation and that both transcriptional and posttranscriptional effects of 5-Aza contribute to increased fetal hemoglobin (HbF) levels.1 While it may be a fine point, the first conclusion was not based on the inability of siRNA-mediated down regulation of DNMT1 to induce fetal globin gene expression, but on the inability of promoter and global DNA demethylation caused by DNMT1 siRNA knock-down to increase levels of fetal globin mRNA and HbF. This conclusion was also based on data showing that a greater decrease in γ-globin promoter and global methylation produced by stably expressed DNMT1 shRNA also failed to induce fetal gene expression and that 5-Aza (at doses that induced near-maximal fetal globin gene expression) caused a localized demethylation of the upstream promoter region but not of downstream or global CpGs. A similar result has been previously presented.2 Since the publication of our manuscript, Fathallah et al have reported that butyrate induction of HbF is also associated with γ-globin promoter hypomethylation.3 This finding is consistent with the idea that promoter demethylation can be a secondary effect and not the primary cause of γ-globin gene induction. While we agree that the maximal hypomethylating effect in the DNMT1 shRNA experiment occurred near the end of differentiation, both the siRNA and shRNA experiments produced hypomethylation equivalent to that seen with effective doses of 5-Aza earlier in differentiation. The fact that Lavelle and colleagues observed higher levels of promoter demethylation with decitabine in baboons is not inconsistent with our observations. Dose-response analyses in our system demonstrated that robust γ-globin gene induction occurred at concentrations of 5-Aza that are insufficient to cause downstream promoter or global DNA hypomethylation. Baboon erythropoietic cells may have been exposed to levels of decitabine that caused both γ-globin induction and higher levels of demethylation. Lavelle et al also take issue with our suggestion that 5-Aza incorporation into mRNA might explain the posttranscriptional effects we observed with the drug. This was only one of several possible alternative mechanisms that we discussed. Because we had not yet determined whether decitabine also exhibits posttranscriptional effects in our system, it would have been premature to rule out this hypothesis. Finally, we never stated or meant to imply that changes in γ-globin promoter methylation during the pharmacologic induction of fetal Hb are unimportant. Even if reduction of γ-globin promoter methylation is not the primary cause of fetal globin gene induction, it is still likely to be an important component of the induction process. 5-Aza and decitabine are potent, clinically active inducers of fetal hemoglobin. Determining the mechanisms of action of these compounds is likely to be an important step in the development of improved pharmacologic strategies for the β-hemoglobinopathies. Only further testing will determine whether our ideas concerning 5-Aza’s mechanisms of action are correct. We hope that our report encourages other investigators to consider these issues.

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


To the editor:

Predicting outcome in childhood acute lymphoblastic leukemia using gene expression profiling: Prognostication or protocol selection?

Flotho et al1 identified within a cohort of 286 childhood acute lymphoblastic leukemia (ALL) patients a simple set of 14 genes (15 Affymetrix probes) with expression in diagnostic bone marrow aspirates that was an independent predictor for patient outcome. It was asked by the authors “how could the genes showing independent prognostic significance in this study be effectively incorporated into existing systems of risk classification?”

We examined the same 15 probe-sets from Affymetrix HU133A–derived gene expression data from 127 ALL patients at diagnosis generated within The Children’s Hospital at Westmead. As per the original report, hierarchical clustering (Figure 1A) illustrates that the 15 probe-set classifier distinguished 2 groups of patients in the same manner as the original paper. Principal component analysis (Figure 1B) indicated that the 2 subpopulations of patients, while distinguishable, were considerably more intermingled than reported by Flotho et al.1 We included the data from 10 bone marrow aspirates from nonmalignant healthy controls, which clustered together within the proximity of both the low and high expression subgroups. Hence, while our results support the classifier as identifying 2 patient populations, the cumulative incidence of relapse show no statistical difference ($P = .35$) between the 2 groups (Figure 1C).

Given the similarity of the 2 studies, it is unlikely that neither technical nor platform deficiencies form the basis of this discrepancy. Unlike previous studies where gene expression signatures...
identified as distinguishing ALL subtypes showed no differential gene expression in our patient cohort, the expression of the genes in this study fall into 2 distinct subgroups. While the original study indicated that gene expression signatures can be used to identify patients at risk of relapse, it must be remembered that relapse can only be interpreted in the context of the chemotherapy protocol and treatment strategy applied. All patients in the original study were treated on the St Jude Total XIII, XIV, XV protocols, while the patients from The Children’s Hospital at Westmead, Australia, were treated on the Berlin-Frankfurt-Munster (BFM) 95 protocol and on a complimentary protocol, Australian and New Zealand Children’s Haematology and Oncology Group (ANZCHOG) VIII.

Within this context, a “low” gene expression profile, which correlated with a poor outcome in the reported study with approximately 50% relapse, had only a 20% relapse rate in our cohort, indicating that patients with this expression profile may have a better response when treated on the BFM95/ANZCHOG VIII protocols. Conversely, the patients with high expression of the 15 genes, which corresponded to less than 10% failure rate in the original patient group, did not respond well to the German nor Australian treatment protocols. This highlights the biologic diversity of ALL patients, such that the 14 “proliferation” genes identified that distinguish response to Total XIII may not be central to the response to other chemotherapeutic protocols.

This validation study demonstrates that the power of gene expression signatures to the clinical management of pediatric ALL may not be related to their diagnosis, subclassification, or indeed prognostication. Rather, our data supports the tenets of the final line in the Flotho et al report; that gene expression profiles may facilitate the “selection of appropriate therapy.” With personalized therapeutics being highly sought after, the value of interpretation of gene expression data with selecting appropriate treatment protocols for patients should not be underestimated.

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References

Response

Genes associated with response to therapy in childhood acute lymphoblastic leukemia

We thank Dr Catchpoole and colleagues for examining the set of 14 genes that we recently reported. These genes were among those significantly associated with minimal residual disease (MRD) during remission induction therapy in children with acute lymphoblastic leukemia (ALL); they were also independent predictors of relapse in a separate patient cohort enrolled in...
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