PROCEEDINGS ARTICLE

Biology of CML stem cells: the basis for clinical heterogeneity?

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Although chronic myeloid leukemia (CML) is now defined on the basis of the presence of the *BCR-ABL1* fusion gene, which may or may not be the initial genetic event that triggers the inappropriate expansion of the myeloid cell mass, CML, similar to other leukemias, is in fact clinically heterogeneous. The biological basis for this heterogeneity is unknown. Here, we summarize some of the data illustrating this heterogeneity and speculate about possible mechanisms that may cause it. It could, for example, be intrinsic in the leukemia stem cell or could be related to some aspect of the patient's response to the leukemia.

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INTRODUCTION

The presence of a *BCR-ABL1* fusion, usually in conjunction with a raised leukocyte count and other less specific clinical and hematological features, defines chronic myeloid leukemia (CML). Because the *BCR-ABL1* gene is a very consistent finding, one might have expected the leukemia to run a very similar course in different patients, but this is conspicuously not the case. This conclusion is based both on clinical observations and the results of laboratory studies, which are explained further in the paper. If the notion of intrinsic heterogeneity is accepted, there are a number of possible causes that may act independently or in various combinations (Table 1). Perhaps the most obvious, which will not be considered further in this paper, is the possibility that there exists a molecular event that precedes and predisposes to the acquisition of the *BCR-ABL* fusion gene; one would also have to postulate that this preceding lesion, for which there is only circumstantial evidence available from a study of a limited number of patients,^{1,2} also influences the clinical expression of the leukemia. Another possibility, which will also not be considered here, is that the *BCR-ABL1*-positive clone has the propensity to acquire new genetic events, presumably as a result of 'genetic instability', and that the degree of this genetic instability varies from patient to patient. The notion that the *BCR-ABL1*-positive clone is in fact predisposed to further genetic change is indeed widely accepted, although there is no evidence for any differing degrees of genetic instability in different patients.

CLINICAL EVIDENCE FOR HETEROGENEITY

The variable clinical course of patients with Ph⁺ CML has been known for many years.^{3–5} In 1984, Sokal *et al.*⁶ studied 613 patients with non-blastic CML collected from six different specialist centers on both sides of the Atlantic. They had been treated predominantly with busulfan.⁶ The authors identified criteria definable at diagnosis, namely age, spleen size, blast cell percentage and platelet numbers, which enabled them to classify the patients into one of three main categories, those with relatively 'good', with 'intermediate' and with relatively 'poor' prognoses. It is notable that this prognostic classification has validity also in patients treated primarily with interferon alpha and for those treated more recently with imatinib.^{7,8} The prognostic

value of the Sokal score could be explained in part by postulating that the precise point within chronic phase when the diagnosis was established impacts on the overall survival measured from diagnosis, but the notion of variation in the leukemia stem cell or in the patient's response to leukemia is not thereby excluded.

Perhaps the best evidence in favor of patient heterogeneity in the era of tyrosine kinases inhibitors (TKI) is the observation that the imatinib response rate is broadly similar in different trials carried out in different countries. Thus, the percentage of patients in complete cytogenetic response and still taking imatinib at 5 years after diagnosis is of the order of 65%, which is generally attributable to the fact that about 20% of patients have proved to be resistant to imatinib and perhaps 15% of patients were intolerant.^{9,10} It seems likely that the proportion of patients who prove to be resistant to second-generation TKI, namely dasatinib or nilotinib, will prove to be lower than those resistant to imatinib, but probably the proportions will be similar in different clinical series using the same drug.

LABORATORY STUDIES

There are multiple possible factors that may affect the absorption, intracellular transport and metabolism of the TKIs; these have been well reviewed by Apperley¹¹ and will not be considered further in this paper. What is required is the identification of leukemia cell-specific factors or patient-specific factors that might impact on the patient's survival regardless of the therapy he or she receives.

One must consider first that the clinical heterogeneity observed in different patients reflects biological diversity that exists in normal persons. Gordon *et al.*¹² addressed the issue in 2003. They used a colony-replating assay to measure the proliferative capacity of myeloid progenitor cells in 211 CML patients and 86 normal persons. Results were expressed as the frequency distributions of the proliferative index (PI) for individual cases. PI values varied among normal individuals but were reproducible in any one person. The PIs from CML patients were significantly greater than that of the normal controls, consistent with increased CML progenitor cell proliferation. It was notable that patients with higher (poorer) scores (according to either Sokal or Hasford

Table 1. Clinical evidence for heterogeneity and possible mechanisms*Clinical*

Sokal *et al.* (1984)⁶
 Hasford (1997)²⁵
 Varying response to TKI—Eutos (2011)⁸

Possible underlying mechanisms

Variations in host factors, immune responses
 'Normal' polymorphisms, e.g., of *BIM* gene
 Environmental factors
 Variations in leukemia cells, e.g., differential use of signal transduction pathways or epigenetic variations

All the mechanisms above could result in variable responses to therapy.

systems) had significantly higher PIs than those with more favorable lower scores. The authors concluded that the heterogeneity in CML patients could be a reflection of the heterogeneity in normal subjects, but the basis of this heterogeneity remained unknown. Support for the notion that clinical heterogeneity may reflect heterogeneity in disease in the corresponding normal population comes from observations that similar heterogeneity may exist in nonmalignant conditions with a single well-defined genetic mutation, such as thalassemia, sickle cell disease and Gaucher's disease.

An interesting example of a constitutional variation that affects a patient's response to imatinib has recently been reported by Ng *et al.*¹³ They found that 12.3% of CML patients of south-east Asian origin had a deletion in the *BIM* gene that was associated with resistance to imatinib. Although the *BIM* deletion may not be relevant to other agents used to manage CML, it could well serve as a model for a constitutional genetic variation that determines heterogeneity in response to other antileukemic agents.

GENE EXPRESSION PROFILING

A considerable number of gene expression studies have been conducted in CML patients in recent years—three are worth mentioning in some detail. Radich *et al.*¹⁴ studied a total of 91 patients in different phases of CML. They identified 3500 genes differentially expressed in the different phases of CML. When patients progressed they observed upregulation of genes associated with ribosomal function and Wnt signaling and downregulation of genes associated with sugar metabolism, myeloid differentiation, apoptosis and DNA damage response. They also observed that patients whose apparently chronic phase disease responded poorly to imatinib had gene expression profiles that resembled advanced-phase rather than chronic-phase disease. They did not set out specifically to examine differences within the chronic-phase population. Subsequently, the same group used a Bayesian model averaging method to identify a 6-gene 'signature' that discriminated chronic phase from blastic crisis and was also able to distinguish between early and late chronic phase. This approach could also prove useful to categorize individual patients at diagnosis and possibly predict their response to TKIs.¹⁵

Yong *et al.*¹⁶ were able to study CD34+ cells collected at diagnosis from two distinct cohorts of CML patients, in one of which blast transformation developed within 3 years of diagnosis and in the other patients remained in chronic phase for 7 or more years. They identified 20 genes differentially expressed in the patients with the 'aggressive' disease compared with those with the more 'indolent' disease. They found that low expression of CD7 and high expression of proteinase 3 or elastase were predictors of longer survival in CML.

Figure 1. Diagrammatic representation of the various points at which epigenetic mechanisms may act to regulate gene transcription (reproduced from AACR Cancer Research Human Epigenome Task Force, *Nature* 2008; 454: 711–755).²⁶ The principal epigenetic mechanisms are DNA methylation, histone tail modifications, chromatin remodelling and non-coding RNA (mainly, microRNAs).

McWeeney *et al.*¹⁷ performed gene expression profiling on CML patients with a view to identifying biomarkers that predicted for imatinib failure. Initially, they identified 885 probe sets with differential expression between responders and nonresponders, but they were able to reduce this number to a classifier subset of 75 genes that successfully identified nonresponders in a validation set. However, this study was not designed to examine differential gene expression profiles in a set of newly diagnosed chronic phase patients.

In general, gene expression patterns may indeed prove valuable in identifying a population of patients who will respond well to TKIs, but such profiles may not, in and of themselves, explain the heterogeneity within a population of CML patients. One might surmise that such heterogeneity was based on fundamental differences in the leukemia cell population between different patients and should have a primary genetic or epigenetic basis.

EPIGENETIC CHANGES

Epigenetic events that could influence gene expression include differential DNA methylation (Table 1), histone tail modifications, chromatin remodeling and aberrant expression of microRNAs (miRs; Figure 1). There are still relatively few reports of DNA methylation status in CML and the majority of these have addressed relatively small numbers of preselected genes. More-

over, most published studies have addressed possible differences between chronic phase and advanced phases; aberrant DNA methylation in a number of genes has been implicated in the progression to advanced phases, including *ABL1*, *CALC1* and *HIC1*.¹⁸ The *HOXA4* gene is frequently hypermethylated in CML and in lymphoid malignancies and may be associated with poor prognosis.¹⁹ Conversely, it appears that downregulation of *BIM* expression is associated with reduced optimal responses to imatinib,²⁰ a finding that links to some extent with the negative effect of *BIM* deletions. By using a methylated CgP island recovery assay, Dunwell *et al.*²¹ identified two genes (*TFAP2* and *EBF2*) that showed increased methylation in chronic phase. Jelinek *et al.*²² studied the methylation status of 10 selected genes. The average percentage of methylated genes was higher in advanced phase than in chronic phase, and abnormal methylation of a *SRC* suppressor gene *PDLIM4* was associated with shortened survival independently of CML phase and imatinib responsiveness.

Although the potential role of histone acetyl transferases and histone deacetylases has been studied extensively in acute leukemia, there are thus far few reports that address CML. Similarly, miR expression has not been extensively studied in CML. One study reported upregulation of *miR-191* and downregulation of *miR 18*, other miR in patients resistant to imatinib.²³ Again, the study was not designed to examine heterogeneity in an unselected population of CML patients. Another more recent study identified differences in miR expression in different phases of CML, but the most notable finding was downregulation of three specific genes, *miR-103*, *miR-150* and *miR-451*. *miR-150* levels were reduced at diagnosis, in hematological response and in blastic transformation, but normal levels were observed in patients receiving imatinib, both those in major molecular response and those who failed treatment. The authors speculated that *miR-150* levels could be inversely related to total leukocyte counts, to levels of BCR-ABL expression or perhaps to both.²⁴

In conclusion, it seems clear that despite the consistent finding of a *BCR-ABL1* gene in CML, individuals differ very substantially in the natural history of their leukemias and in their response to treatment. These differences may relate to variations intrinsic in the leukemia cell or in the environment in which they proliferate, or to some combination of the two. Elucidating the mechanisms that underlie this heterogeneity is of fundamental importance, because it may facilitate selection of the best treatment for a given patient and because it may help to explain the heterogeneity seen in other forms of leukemia and in other malignant diseases.

CONFLICT OF INTEREST

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