Clinical Applications of Molecular Haematology: An Overview

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Abstract

Haematological disorders are getting more and more molecularly defined. This is specially so in the field of haematological malignancies. The molecular understanding has helped in fine-tuning the diagnosis, prognosis and management. The tests are becoming widely available and they enjoy both sensitivity and specificity. Following is an overview of clinical applications of molecular haematology, especially in the field of chronic myeloid leukaemia, chronic eosinophilic leukaemia, bcr abl negative chronic myeloproliferative disorders and acute promyelocytic leukaemia.

INTRODUCTION

Molecular haematology has contributed to major advances in diagnosing, classifying and treating myeloid neoplasms. These advances have been in the field of acute myeloid leukaemia and the chronic myeloproliferative disorders with little advance yet in the myelodysplastic syndromes. Basic and clinical research relating to the BCR-ABL, FIP1L1-PDGFRA, ETV6-PDGFRA, ZNF198-FGFR1 and PML-RARA fusion genes and the V617F point mutation in JAK2 illustrate these advances.

Our modern understanding of myeloid neoplasms started as far back as the middle of the 19th century when chronic myeloid leukaemia (CML) was first recognised by post-mortem macroscopic and microscopic examination of the blood.1 Our progress in understanding and effectively treating this disease provides an example of how haematological knowledge, at first based on morphology, and then supplemented by cytochemistry and cytogenetic analysis and was finally deepened with the application of molecular genetic techniques (Fig. 1). Each new technique applied has, in general, not supplanted earlier methodologies but has supplemented them to enrich our knowledge. Therapy of CML was initially based on empirical evidence, which supported the use of arsenic and benzene, although not perhaps purgatives, potassium iodide, quinine, the application of leeches and other early forms of treatment. Increasingly, methods of assessing the results of treatment became more rigorous and scientifically based until finally, with the advent of tyrosine kinase inhibitors such as imatinib, scientific knowledge, in the form of molecular genetics, also led to the development as well as the validation of novel forms of therapy (Fig. 1).2

A similar trajectory from morphology to cytogenetic and molecular genetic analysis has been followed, somewhat later, in other myeloproliferative disorders and in the acute myeloid leukaemias. The myelodysplastic syndromes have lagged behind, still being largely refractory to a molecular understanding. In the case of the acute myeloid leukaemias, immunophenotyping has also had a role but this has been of only minor importance in the more chronic disorders.

Molecular genetic techniques are now crucial in assessing response to treatment as well as in diagnosis and classification. Increasingly also, molecular techniques are likely to lead to the development of
effective drugs directed at molecular targets.

The contribution of molecular haematology in myeloid neoplasms is best exemplified by consideration of chronic myeloid leukaemia, chronic eosinophilic leukaemias, the JAK2-related myeloproliferative disorders and acute promyelocytic leukaemia. The techniques now available have yielded a great deal of information but are only valid when interpreted in a clinical and haematological context. The BCR-ABL1 fusion gene, for example, is found not only in chronic myeloid leukaemia in all its phases but also in Ph-positive essential thrombocytosis and acute leukaemia—lymphoblastic, myeloid and biphenotypic.

**Chronic Myeloid Leukaemia**

Cytogenetic and molecular techniques have demonstrated that chronic myeloid leukaemia (CML) is a unique disorder. Such techniques identified the cell of origin as a pluripotent myeloid-lymphoid stem cell and demonstrated that further mutations occurred during the accelerated and acute phases of the disease. When applied to patients being treated with cytotoxic drugs such as busulphan and hydroxyurea (previously hydroxyurea), genetic techniques demonstrated that although haematological remission occurred the leukemic clone persisted. Conversely when applied to patients treated by stem cell transplantation or, occasionally, interferon they demonstrated that the leukemic clone could disappear and that there was therefore the possibility of elimination of disease. Following transplantation, molecular techniques permitted monitoring of residual disease and led to the early application of donor leucocyte infusions in such patients before overt haematological relapse occurred. Finally, demonstration that the BCR-ABL1 fusion gene encoded a constitutively activated tyrosine kinase led to a drug discovery exercise that yielded imatinib, a fairly specific inhibitor of this kinase. Molecular investigation of the basis of imatinib resistance revealed that this was sometimes due to further mutation in the BCR-ABL1 gene and led in turn to development of more powerful tyrosine kinase inhibitors, such as dasatinib and nilotinib, which were also able to inhibit most of the tyrosine kinases encoded by BCR-ABL1 genes with further mutations.

Imatinib has revolutionised the treatment not only of CML and BCR-ABL1-positive acute leukaemias but also that of gastrointestinal stromal cell tumours with a KIT mutation and certain subtypes of eosinophilic leukaemia with mutations in PDGFRα and PDGFRβ (see below). Rare patients with systemic mastocytosis and an uncommon V560G KIT mutation also benefit but the majority of systemic mastocytosis patients with a D816V KIT mutation do not benefit.

**Chronic Eosinophilic Leukaemia**

In the past, the diagnosis of chronic eosinophilic leukaemia was often difficult and was sometimes only made in retrospect when acute transformation occurred. Sometimes the diagnosis could be made in the chronic phase when a clonal cytogenetic abnormality was demonstrated but in other patients no such abnormality was present and the diagnosis remained uncertain. In the last five years many patients in whom the diagnosis would previously have been ‘idiopathic hypereosinophilic syndrome’ have been found, by the application of molecular techniques, to have a neoplastic condition. A common theme in patients with chronic eosinophilic leukaemia and other myeloproliferative/myelodysplastic syndromes with prominent eosinophilia is the presence of a mutation in either PDGFRα or PDGFRβ. The two most typical molecular lesions are: (i) a FIP1L1-PDGFRα fusion gene occurring as a result of a cryptic interstitial deletion at 4q12 or (ii) an ETV6-PDGFRβ fusion gene occurring as a result of t(5;12)(q33;p13). There are also rare variants in which either PDGFRα or PDGFRβ is fused to a different partner gene. Disease associated with t(5;12)(q33;p13) can be recognised by standard cytogenetic techniques but recognition of cryptic del4(q12) and FIP1L1-PDGFRα fusion requires the application of molecular techniques, either fluorescence in situ hybridisation (FISH) or the polymerase chain reaction (PCR); sometimes the extra sensitivity of nested PCR is needed. Recognition of these two uncommon syndromes is important because both are responsive to imatinib.

Another rare subtype of chronic eosinophilic leukaemia is that associated with one of three fusion genes incorporating part of FGFR1, either a ZNF198-FGFR1 fusion resulting from t(8;13)(p11;q11-12), a FOP(FGFR1OP)-FGFR1 fusion resulting from t(6;8)(q27;p11) or a CEP1-FGFR1 fusion resulting from t(8;9)(p11-12;q33). In addition to these three mutations there are a number of very rare instances of patients with other fusion genes incorporating part of FGFR1. This syndrome, sometimes designated the 8p11 stem cell syndrome, includes not only chronic eosinophilic leukaemia but also acute myeloid leukaemia and acute lymphoblastic leukaemia/lymphoma, mainly but not only of T lineage. As for CML, it has been possible to demonstrate the genetic abnormality in cells of lymphoid and myeloid lineages indicating that the responsible mutation occurred in a pluripotent stem cell. It is likely that, as for the leukaemias associated with PDGFRα and PDGFRβ, it will be possible to develop or identify a specific tyrosine kinase inhibitor that will be effective in this syndrome. Preliminary evidence (mouse model and a single patient) suggests that PKC412 may be efficacious.

**JAK2-Mutated Myeloproliferative**
DISORDERS

The almost simultaneous identification of a JAK2 V617F (exon 14) mutation by five groups9,10 has started to reveal the molecular basis of Ph-negative classical myeloproliferative disorders. This mutation is found in about 95% of patients with polycythaemia vera and in about half of patients with essential thrombocythaemia and myelofibrosis (both de novo idiopathic myelofibrosis and that following polycythaemia vera or essential thrombocytois). Interestingly many patients with polycythaemia are homozygous for JAK2 V617F as a result of mitotic recombination having led to uniparenteral disomy. Patients with essential thrombocythaemia found to have this mutation have some disease characteristics more similar to those of polycythaemia vera than is the case in patients without the mutation, for example a higher haemoglobin concentration and a higher white cell count. The few patients with polycythaemia who do not have a JAK2 V617F mutation often have a different mutation in the same gene, one of a number of different mutations in exon 12.14 These patients have somewhat different disease characteristics from classical polycythaemia vera in that the platelet count and the white cell count are usually normal or near normal; without the discovery of the mutation many of them would have been classified as having idiopathic erythrocytosis.

The JAK2 V617F mutation is present in a significant proportion of patients with a myelodysplastic/myeloproliferative disorder with sideroblastic erythropaiesis and thrombocytosis, suggesting that this syndrome may be more closely related to the myeloproliferative disorders than to the myelodysplastic syndromes.15

The JAK2 V617F mutation has also been found in a significant proportion of patients with portal, splenic or splanchnic vein thrombosis.16 If such patients do not have disease characteristics that permit recognition of one of the classical myeloproliferative disorders, the disease should be designated ‘myeloproliferative disorder, unclassified’.

JAK2 mutations will be incorporated into the revised World Health Organisation classification of haematological neoplasms. The possibility of demonstrating this mutation will reduce the need for other tests.

Acute promyelocytic leukaemia

Acute promyelocytic leukaemia is just one of many subtypes of acute leukaemia that can be recognised as entities partly on the basis of a specific genetic abnormality. There are three morphological variants—classical, hypogranular (or microgranular) and hyperbasophilic. All are morphologically recognisable although only in the classical hypergranular form is recognition absolutely straightforward. All type of acute promyelocytic leukaemia have the same cytogenetic and molecular genetic abnormality, t(15;17)(q22;q21) and a PML-RARA fusion gene, which are predictive of responsiveness to all-trans retinoic acid (ATRA) and arsenic trioxide (As$_2$O$_3$). It is important to distinguish acute promyelocytic leukaemia from acute leukaemia associated with t(11;17) (q23;q21) and a PLZF-RARA fusion gene, which is morphologically somewhat different (often with less striking arrest at the promyelocyte stage and less hypergranular) and is also refractory to ATRA and As$_2$O$_3$. There are also other molecular variants with other fusion genes involving RARA, which should be distinguished.

As with other molecularly defined entities, the PML-RARA fusion gene can be used not only in diagnosis but also to monitor minimal residual disease in order to alter treatment if there is a rising level of transcript from the fusion gene.

CONCLUSION

Haematology is increasingly incorporating molecular information. In the case of haematological neoplasms such information is used both in diagnosis and management. Some haematological neoplasms can be diagnosed reliably only when molecular genetic techniques are employed.

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