Epigenetic Therapy Comes of Age: Waking Up Silenced Tumour Suppressor Genes in Myelodysplastic Syndrome

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Myelodysplastic Syndrome

Myelodysplastic Syndrome (MDS) is a group of clonal stem cell disorders with ineffective haemopoiesis of the bone marrow.1-2 Clinically MDS is characterised by peripheral cytopenia with a hypercellular and dysplastic bone marrow. Patients usually present with complications of cytopenia including symptomatic anaemia, bleeding from thrombocytopenia or infections from neutropenia. MDS comprises a group of disorders with variable cytopenia and a variable amount of myeloblasts, and hence a variable risk of leukaemic transformation. In French-American-British (FAB) classification,3 MDS comprises 5 disorders based on the severity of cellular dysplasia, the nature of erythroid dysplasia, the amount of blast cells and presence of monocyties including refractory anaemia (RA), refractory anaemia with ringed sideroblasts (RARS), chronic myelomonocytic anaemia (CMML), refractory anaemia with excess blasts (RAEB) and refractory anaemia with excess blasts in transformation (RAEB-t).3 Frequent karyotypic aberrations include chromosomal loss such del(5q) or 5q-, del(7q) 7q- or chromosomal gain such as trisomy 8. In general, normal karyotype or 5q- are associated with good prognosis while complex karyotypic abnormality and chromosomal loss of 7q- with poor prognosis. Moreover, based on the amount of blasts, severity of cytopenia and the type of chromosomal aberration, patients with MDS can be stratified into different risk groups by the International Prognostic Scoring System (Table 1).4 In general, based on the median survivals, MDS can be classified into high-risk or low-risk with median survivals <5 or >5 years. High-risk MDS include RAEB and RAEB-t and low-risk MDS includes RA or RARS. (Figure 1)

Treatment may be directed towards killing the blasts by conventional chemotherapy (such as cytarabine and daunorubicin used in acute myeloid leukaemia) or ameliorating the pancytopenia by cytokine therapy such G-CSF or erythropoietin. However, the only modality of treatment that may render a cure is bone marrow transplantation (BMT) in the form of high-dose therapy with allogeneic haemopoietic stem cell rescue (allo-BMT). Recently, the advent of non-myeloablative allogeneic BMT or mini-allo-BMT has allowed more elderly MDS patients to receive BMT.5 On the other hand, certain MDS subtypes may benefit from other therapies. For instance, patients with sideroblastic anaemia may respond to high-dose of pyridoxine and patients with hypoplastic MDS may respond to immunosuppressive therapy such as cyclosporine A or anti-thymocyte globulin (ATG).5 A special syndrome of MDS, 5q-syndrome, in which 5q- is the sole cytogenetic aberration, is characterised by refractory anaemia with thrombocytosis in a middle or old-aged female.6 Interestingly, patients with 5q- syndrome respond favourably to an immunomodulatory agent, lenalidomide, which is a derivative of thalidomide. However, 5q- syndrome is an uncommon subtype amongst MDS. Despite the large number of treatment options, the majority including conventional chemotherapy are largely palliative, and do not lead to a cure. Indeed, the majority of the current treatments may result in improvement in blood counts but do not alter the natural history of the disease especially leukaemic transformation. Moreover, while allogeneic BMT is potentially curative, only young patients with an HLA-identical sibling can be considered because of the inherent risk of graft-versus-host disease and infective complications, and hence is not applicable to the majority of elderly MDS patients.7 Therefore, an alternative treatment strategy especially one that may reduce the risk of leukaemic transformation is urgently needed.

DNA Methylation

DNA methylation, catalysed by DNA methyltransferase, involves the addition of a methyl group to the carbon 5 position of the cytosine ring in the CpG dinucleotide and results in the generation of methylcytosine.8,9 (Figure 2) Methylation of cytosine to methylcytosine in DNA is a heritable genetic alteration during cell replication in the absence of any change in the genetic sequence. In the normal mammalian genome, CpG rich regions (CpG islands) exist and these are often found within the promoter of genes. These promoter-associated CpG islands that serve as gene transcription-ready state.8,9 The only exceptions are the promoters of selectively silenced alleles in imprinted autosomal genes, and the gene promoters of the inactivated X-chromosome of females. By contrast, CpG islands of various genes have been shown to be...
aberrantly methylated (hypermethylated) in cancer. Importantly, hypermethylation of gene promoters has been shown to result in repression of gene transcription and gene silencing, thus serving as an alternative mechanism of gene inactivation. The mechanism of gene silencing has recently been shown to be related to the recruitment of repressor protein complex containing histone deacetylase and other repressor proteins such as methyl-cytosine binding protein (MBP), resulting in deacetylation of histone covered by the hypermethylated promoter DNA. This results in a closed chromatin structure that precludes access of the active transcription complex and hence gene silencing. However, the mechanism of these de novo gene promoter hypermethylation is largely unknown, and is the topic of intensive research.

Promoter Hypermethylation in Haemic Malignancies

In haemic malignancies, methylation of tumour suppressor genes including CDKN2B (alias P15), CDKN2A (alias, P16), P73, DAP kinase, SHP1 has been reported in various haematological malignancies.9-19 These genes either regulate progression of the cell cycle (CDKN2A and B), and hence cellular proliferation, the induction of apoptosis upon detection of oncogenic transformation (P14, P73 and DAP kinase) or intracellular JAK/STAT signalling such as SHP1.9-19 For instance, P15 but not P16 is frequently methylated in acute leukemia but both P15 and P16 are frequently methylated in NHL, MM and CLL. SHP1 is frequently methylated in literally all types of haemic cancers but p73 only methylated in Burkitt’s lymphoma. Therefore, there is heterogeneity in the profile of gene methylation in different types of haemic malignancies. Importantly, re-expression of these genes has been shown in vitro by 5-AzaCytidine treatment to result in growth inhibition and/or apoptosis. Moreover, the high frequency of methylation of certain genes in some haemic malignancies suggests that gene hypermethylations are probably early events in the pathogenesis of these cancers. For instance, P15 is methylated in >70% of acute leukemia including APL, which carries the PML-RARA fusion gene, suggesting p15 gene methylation might collaborate with t(15;17) in leukaemogenesis.15 Similarly, in mantle cell lymphoma with upregulation of cyclin D1, SHP1 has been shown to be methylated in >80% of cases, suggesting that SHP1 methylation might be an early event collaboration with cyclin D1 dysregulation in lymphomogenesis. On the other hand, methylation of some genes is associated with disease progression, e.g. P16 methylation at relapse but not diagnosis in acute leukaemia,16 and Abl methylation during progression to accelerated phase or blastic transformation in CML. Furthermore, certain methylated genes are associated with prognosis and survival. For instance, P15 methylation was shown to confer an inferior DFS in APL.13,15,16 Therefore, aberrant gene promoter methylation is potentially important in either pathogenesis, progression and prognosis. Therefore, treatments which may reverse these methylation alterations are potentially beneficial in haematological cancers.

Therapeutic DNA Methyltransferase Inhibitors

In clinical practice, two cytidine analogues, azacytidine (5-azacytidine; Vidaza Pharmion, USA) and Decitabine (5-aza-2’-deoxycytidine; DCB, Decogen, SuperGen, USA), have been shown to carry hypomethylating properties by inhibiting DNA methyltransferase.20 In cancers, inhibition of DNA methylation reactivates the expression of tumour suppressor genes that have undergone epigenetic silencing, and leads to apoptosis of cancer cells.

In MDS, CDKN2B (alias, P15), a cyclin-dependent kinase inhibitor that negatively regulates the cell cycle and hence cellular proliferation, has been shown to be hypermethylated in marrow stem (CD34+) cells in patients with MDS,21 and is potentially important in its pathogenesis. Therefore, clinical trials have been conducted to test the efficacy of these DNA methyltransferase (DNMT) inhibitors in MDS, and hence the concept of hypomethylating therapy. At present both Vidaza and Decitabine are approved for the treatment of MDS.

Vidaza in MDS

After promising results from 2 phase II studies by the CALGB in patients with RAEB, RAEB-T and CMML, a phase III study using Vidaza in the treatment of MDS has been published in 2002.22 Recently, data from a phase III Cancer and Leukaemia Group B (CALGB) 9221 study led to the approval of Vidaza by the US Food & Drug Administration (FDA). In the study, MDS patients were randomised to receive Vidaza and best supportive care. Vidaza was given subcutaneously at the dose of 75mg/d x 7 days at 28-day cycles. Moreover, patients in the BSC arm in whom the disease progressed might cross-over to receive Vidaza after 4 months. Responses (complete, partial or haematological improvement) were assessed after 4 cycles of treatment. Patients in complete remission would receive 3 further cycles of Vidaza treatment. 191 patients were recruited. There was a significant improvement in overall response in the Vidaza arm compared with the BSC arm (ORR 60% Vidaza arm versus 5% BSC arm, p=0.001). Complete and partial remissions occurred only in the Vidaza but not the BSC arm. The frequency of leukaemic transformation was also significantly reduced in the Vidaza arm (15% versus 38%). Because of the cross-over nature of the study, a landmark study was conducted to assess the impact of Vidaza treatment on survival, which showed median survival of 18 and 6 months in the Vidaza and BSC arms (p=0.03). Moreover, the improved survival was associated with an improvement in the quality of life.

Decitabine in MDS

On the other hand, phase I/II studies of decitabine (DCB) in high-grade MDS has also been conducted.23 O’Brien et al showed that in 52 patients with high-grade MDS, an overall response was observed in 81% of patients with 35% CR rate. Wijermans et al showed that in 66 patients with intermediate- to high-grade MDS,
DCB (at 45mg/m²/d x 3 days every 6 weeks), overall response rate was 49% with 20% being CR. These encouraging data confirmed the efficacy of DCB in MDS, which accumulated to a phase III study where 170 patients with MDS were randomised to receive low-dose DCB (15mg/m² every 8 hourly x 3 days, repeated every 6 weeks) or best supportive care (BSC). The study showed a superior overall response rate of 30% (with 9% CR) in the decitabine arm compared with 7% (no CR or PR) in the BSC arm (p<0.001). There was a non-significant delay in leukaemic transformation (time to leukaemia was 12 months in the decitabine arm and 8 months in the BCS arm) but a significant delay in those with high-risk MDS. Moreover, patients treated with decitabine had improved quality of life, and cytogenetic remission was shown in some cases. However, there was no difference in overall survival. On the other hand, comparison with a historical control group of high-grade MDS patients treated with conventional chemotherapy, who were matched in age, sex, cytogenetic findings and international prognostic scoring system with 115 MDS patients treated with low-dose decitabine, showed that there was an obvious overall survival advantage in high-grade MDS patients receiving decitabine (median overall survival: 22 months versus 12 months, p<0.001). Therefore, possible survival advantages may be detected in future prospective trials. Moreover, there are recent data that patients who progressed or failed to respond to Vidaza did respond to decitabine.

Common Features in Both DNMT Inhibitor Trials

First, a response was only demonstrated after several cycles of treatment. Therefore, had the patients been considered non-responding and taken off study after 2 cycles, the response would not have been captured. For instance, the median time to response was > 3 cycles in the CALGB9221 trial, and > 2 cycles in the Decitabine trial. Only CR or PR occurred in the DNMT inhibitor arm while only soft end-point such as haematological improvement could occur in the best supportive care arm. Both studies were associated with improved QOL in patients receiving DNMT inhibitors. Moreover, delay in leukaemia transformation was observed in the Vidaza trial, and in the subgroup of high-risk MDS in the Decitabine trial. Major side-effects from these DNMT inhibitors (Vidaza & decitabine) were worsening cytopenia.

Future

Vidaza can be administered in an out-patient setting, and has been shown to be effective in all MDS subtypes, and results in delay of leukaemic transformation, and likely improvement in overall survival. Decitabine is an alternative to Vidaza but impact on survival remains to be seen in further analysis of the phase III study.

On the other hand, from the mechanistic point of view, gene silencing from promoter hypermethylation is enhanced by further modification of histone molecules, primarily deacetylation of the regional histone molecules, where the stretch of hypermethylated promoter DNA covers. Therefore, one would anticipate a synergistic effect if histone deacetylase inhibitors are added to these DNMT inhibitors so that both DNA methylation and histone deacetylation are reversed, and hence render an open chromatin in the promoter concerned, and allow access of transcription complex to the gene promoter. Indeed, clinical trials incorporating both DNMT inhibitors and histone deacetylase inhibitors are on-going, and the results are eagerly awaited.

The advent of the hypomethylating treatment in MDS is important in the following ways. First, MDS is a disease of the elderly who generally cannot tolerate intensive chemotherapy, and hence demethylating therapy is particularly appealing as they do not mediate their activity by cytotoxicity. Second, unlike mutations in cancers, which are irreversible, gene promoter hypermethylation is a reversible process, and hence is an important modality of therapy to the treatment of cancers.
Figure 2. shows the binding of the promoter-associated CpG island by a transcription complex in unmethylated promoter, but the exclusion of transcription complex in a promoter that is aberrantly methylated.

References