Effects of the MEK inhibitor CI-1040 (PD 184352) on progenitor growth from normal and myelodysplastic marrow

Previous studies demonstrated that ~50% of acute myelogenous leukemia samples exhibit activation of the mitogen-activated protein kinase (MAPK) pathway and growth inhibition by MEK inhibitors. To extend these observations, we assessed MAPK pathway activation and effects of CI-1040 in normal and myelodysplastic (MDS) marrow.

Activation of the mitogen-activated protein kinase (MAPK) pathway, as manifested by phosphorylation of extracellular signal regulated kinases (ERK) 1 and 2 on a specific Thr-X-Tyr motif, is usually assessed by immunoblotting. Because normal and myeloblastic (MDS) marrow contain mixed cell populations, immunohistochemistry was utilized to assess cell type-specific pathway activation. Under Institutional Review Board-approved protocols, paraffin-embedded marrows from 14 MDS patients (3 RA, 5 RAEB, 3 RCMD, 3 CMML) and 4 controls were treated with 3% H2O2 to inhibit peroxidases, subjected to heat-induced antigen retrieval, and stained with rabbit anti-phospho-ERK1/2 (anti-ACTIVE™ p44/42 MAPK; Promega, Madison, WI, USA) followed by biotinylated secondary antibody and horseradish peroxidase-conjugated streptavidin. Fixed, paraffin-embedded HL-60 leukemia cells and normal human tonsil served as positive and negative controls, respectively. This analysis demonstrated MAPK activation in all MDS marrows examined. As illustrated in Figure 1A, staining was strongest in blast cells and myeloid progenitors, intermediate in erythroid cells, and weakest in megakaryocytes. The staining strength and pattern did not differ among MDS subtypes. In normal marrow, a similar pattern was observed, although the staining intensity was lower (Figure 1B).

In view of these results, the effect of the MEK inhibitor CI-1040 on growth of erythroid and myeloid progenitors in vitro was examined. Fresh marrow from 12 MDS patients, 14 patients with cytopenias but no evidence of MDS, and 3 normal patients undergoing hip arthroplasty, as well as blood from 5 normal volunteers was fractionated on Ficoll-Hypaque gradients. Mononuclear cells (6x10^6/3 mL Methocult™ methylcellulose medium [StemCell Technologies; Vancouver, BC, USA]) containing diluent or 10 µmol/L CI-1040 were plated in 3 mL Methocult™ methylcellulose medium [StemCell Technologies; Vancouver, BC, USA] containing diluent or 10 µmol/L CI-
CI-1040 (Pfizer, Ann Arbor, MI, USA), a concentration that approximates serum levels of CI-1040 and its active metabolite observed in clinical trials. As specified by the manufacturer of the medium, progenitor colonies (summed BFU-E/CFU-E and CFU-GM/CFU-G) were quantified on day 14 using established morphologic criteria. CFU-GEMM colonies were very scarce in all samples and were not enumerated. Results obtained in these assays are summarized in Figure 2. CI-1040 inhibited erythroid colony formation by >50% in 10 of 12 MDS samples (median inhibition 87%), 5 of 8 non-MDS cytopenic samples (median 67%) and 3 of 6 normal samples (median 41%). Inhibition was greater with 10 µM CI-1040 than with 1 µM CI-1040, as expected for a dose-dependent process. The degree of inhibition did not vary with MDS subtype. In addition, as illustrated in Figure 2G and H, CI-1040 inhibited by >80% the extensive blast background (small poorly formed colonies that do not mature or form normal colonies in Methocult™ assays) that was present in 3 diluent-treated MDS samples. In contrast, CI-1040 inhibited myeloid colony formation by >50% in only 3 of 11 MDS samples (median inhibition 15%), 1 of 14 non-MDS cytopenic samples (median 25%), and 0 of 8 normal samples (median 0%).

Although a recent biochemical study demonstrated MAPK pathway activation in erythropoietin-treated erythroid progenitors from MDS marrow, to our knowledge this is the first demonstration of MAPK pathway activation in unstimulated normal and MDS marrow. In the absence of exogenous stimuli, erythroid progenitors consistently stained less intensely than myeloid cells (including undifferentiated blasts). While this might reflect lower levels of MAPK activation by endogenous erythropoietic vs. myelopoietic cytokines, it is also possible that ERK-mediated induction of MAPK phosphatases results in underestimation of pathway stimulation in erythroid cells or that phospho-ERK staining in identifiable progenitors underestimates pathway activation in more primitive erythroid colony-forming cells.

These results also provide the first evidence that clinically achievable CI-1040 concentrations inhibit MDS progenitors. While it is possible that cytokines added to support colony formation activated the MAPK pathway and enhanced CI-1040 sensitivity in vitro, our observation of pathway activation in situ (Figure 1) argues that exogenous cytokines are not activating a normally quiescent pathway. Several observations also suggest that CI-1040 might be inhibiting clonal precursors of MDS in vitro. First, effects of CI-1040 were greater in MDS marrow (Figure 2). Second, a marked reduction of clonally derived blast colonies was observed (Figures 2G,H). Because none of the MDS samples in the present study had informative cytogenetics, study of additional MDS samples is required to confirm the effects on clonal progenitors. Nonetheless, our preliminary results suggest that the MAPK pathway warrants further investigation as a potential drug target in MDS.

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Letters to the Editor

Methodologic and biological variability of quantitative real-time polymerase chain reaction analysis of Bcr-Abl expression in Philadelphia chromosome-positive acute lymphoblastic leukemia

We examined to what extent technical and biological factors may affect the validity of Bcr-Abl polymerase chain reaction quantitation in Philadelphia chromosome-positive acute lymphoblastic leukemia and found that technical variance is the predominant limitation of the method and was not exceeded by biological variance. Interestingly, the number of p210Bcr-Abl transcripts per blast was more than 10-fold higher than the number of p190Bcr-Abl copies per blast.

References


