Characterization of acquired EPZ-5676 resistance in cell line models of MLL rearranged leukemia
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Abstract
DOT1L inhibitor EPZ-5676 is currently under Phase I clinical trial investigation in relapsed/refractory patients with acute leukemia, including those with an MLL-rearrangement (MLL-r). Early clinical results, including complete remissions, support ongoing clinical development and preclinical investigation into mechanisms precipitating EPZ-5676 treatment induced resistance. MLL-r cell lines KOPN-8 (MLL-ENL) and NOMO-1 (MLL-AF9) were exposed to an EPZ-5676 concentration above the pre-determined 14 day proliferation assay IC50. Initial treatment of the cell lines led to the expected inhibition of H3K79 dimethylation (H3K79me2) and MLL-r target genes HOXA9 and MEIS1 as outlined in previous work (3). Resistance to EPZ-5676 in both cell lines emerged following three weeks of continued treatment with EPZ-5676 and was defined by increased growth rates in the presence of inhibitor. Mechanisms of resistance for both cell lines were investigated using RNA-seq and ChIP-seq on parental and resistant cell line pools. Our analysis identified common characteristics between the resistant cell lines, but mechanisms by which they became resistant differed. Global H3K79me2 reduction was maintained in both refractory cell lines, yet ChIP-seq analysis of resistant pools identified specific loci with H3K79me2 recovery in KOPN-8 cells. In resistant KOPN-8 cells recovery of H3K79me2 was concentrated at the HOXA locus and other MLL-r target genes (e.g. MEIS1 and RUNX2), with the remainder of actively transcribed genes maintaining H3K79me2 inhibition at levels observed in parental cell. In contrast, resistant NOMO-1 cells did not recover H3K79me2 at any actively transcribed genes, including those of the MLL-r signature. Only resistant KOPN-8 cells regained expression of the MLL-r target genes actively transcribed genes, including those of the MLL-r signature. Only resistant KOPN-8 cells became resistant differed. Global H3K79me2 reduction was maintained in both refractory cell lines, yet ChIP-seq analysis of resistant pools identified specific loci with H3K79me2 recovery in KOPN-8 cells. In resistant KOPN-8 cells recovery of H3K79me2 was concentrated at the HOXA locus and other MLL-r target genes (e.g. MEIS1 and RUNX2), with the remainder of actively transcribed genes maintaining H3K79me2 inhibition at levels observed in parental cell. In contrast, resistant NOMO-1 cells did not recover H3K79me2 at any actively transcribed genes, including those of the MLL-r signature. Only resistant KOPN-8 cells regained expression of the MLL-r target genes actively transcribed genes, including those of the MLL-r signature. Only resistant KOPN-8 cells became resistant differed. Global H3K79me2 reduction was maintained in both refractory cell lines, yet ChIP-seq analysis of resistant pools identified specific loci with H3K79me2 recovery in KOPN-8 cells. In resistant KOPN-8 cells recovery of H3K79me2 was concentrated at the HOXA locus and other MLL-r target genes (e.g. MEIS1 and RUNX2), with the remainder of actively transcribed genes maintaining H3K79me2 inhibition at levels observed in parental cell. In contrast, resistant NOMO-1 cells did not recover H3K79me2 at any actively transcribed genes, including those of the MLL-r signature. Only resistant KOPN-8 cells regained expression of the MLL-r target genes actively transcribed genes, including those of the MLL-r signature. Only resistant KOPN-8 cells became resistant differed. Global H3K79me2 reduction was maintained in both refractory cell lines, yet ChIP-seq analysis of resistant pools identified specific loci with H3K79me2 recovery in KOPN-8 cells. In resistant KOPN-8 cells recovery of H3K79me2 was concentrated at the HOXA locus and other MLL-r target genes (e.g. MEIS1 and RUNX2), with the remainder of actively transcribed genes maintaining H3K79me2 inhibition at levels observed in parental cell. In contrast, resistant NOMO-1 cells did not recover H3K79me2 at any actively transcribed genes, including those of the MLL-r signature. Only resistant KOPN-8 cells regained expression of the MLL-r target genes actively transcribed genes, including those of the MLL-r signature. Only resistant KOPN-8 cells became resistant differed. Global H3K79me2 reduction was maintained in both refractory cell lines, yet ChIP-seq analysis of resistant pools identified specific loci with H3K79me2 recovery in KOPN-8 cells. In resistant KOPN-8 cells recovery of H3K79me2 was concentrated at the HOXA locus and other MLL-r target genes (e.g. MEIS1 and RUNX2), with the remainder of actively transcribed genes maintaining H3K79me2 inhibition at levels observed in parental cell. In contrast, resistant NOMO-1 cells did not recover H3K79me2 at any actively transcribed genes, including those of the MLL-r signature. Only resistant KOPN-8 cells regained expression of the MLL-r target genes actively transcribed genes, including those of the MLL-r signature.

Results

Figure 6: Analysis of ChIP-seq and RNA-seq Data Based on Inferred Mechanisms

Conclusions
Continuous exposure of two MLL-r cell lines to EPZ-5676 lead to development of a resistant phenotype despite maintenance of global H3K79me2 inhibition

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