

WHOLE EXOME SEQUENCING IDENTIFIES NOVEL MUTATIONS IN RELAPSED OR REFRACTORY ACUTE PROMYELOCYTIC LEUKAEMIA FAILING TREATMENT WITH ORAL ARSENIC TRIOXIDE

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BACKGROUND

Arsenic trioxide (As₂O₃)-based regimens is effective in inducing a remission in more than 90 percent of relapsed acute promyelocytic leukaemia (APL). Treatment failure following As₂O₃ and central nervous system (CNS) involvement is associated a poor prognosis. With the widespread clinical application of arsenic trioxide in the frontline and relapsed setting, arsenic trioxide resistance is an emerging clinical problem. There is paucity of information of the molecular mechanisms underlying resistance to As₂O₃.

AIMS

To determine mutations associated with resistance to oral As₂O₃ in relapsed or refractory APL using serial whole exome sequencing.

METHODS

DNA was extracted from the buffy coat in serially archived bone marrow (BM) samples of 7 patients with 2 or more relapses following treatment with oral As₂O₃. Paired BM samples (As₂O₃-resistant sample was paired with the corresponding As₂O₃-sensitive sample from the same patient) were evaluated with whole exome sequencing (WES) using the illumina HiSeq 1500 platform with an average depth of 100X. The exome data was analysed using bio-informatic pipeline specifically designed to identify single nucleotide variants (SNVs) with increased allele frequency during oral As₂O₃ failure. The functional influences of nonsynonymous missense mutations are evaluated using SIFT, Polyphen2 and FATHMM. To confirm putative SNVs in these samples, re-sequencing by Sanger sequencing was performed using DNA submitted for exome sequencing. The confirmed SNVs were evaluated in the entire validation cohort of 22 patients with relapsed or refractory APL following oral As₂O₃-based treatment. Clinicopathologic features, karyotype, *FLT3* and *PML-RARA* mutations were determined in all patients with relapsed or refractory APL.

RESULTS

By serial WES in 7 patients with APL refractory to oral As₂O₃-based treatment, the following SNVs of function-changing potential had increased allele frequency and during treatment resistance: *NOTCH2*, *FOXD4L5*, *KCNJ11/18*, *MADCAL1*, *CBR3*, *NSD1*, *FLG1*, *CCDC179*, *SIGLEC11*, *ROBO4*, *CISD2*, *WT1*, *PTCH1*, *KMT2D* and *MED17*. *PML* B2 domain mutations were not seen. These putative SNVs were all confirmed positive by Sanger sequencing. The frequencies of the mutations were validated in a cohort of 22 patients with relapsed or refractory APL following oral As₂O₃-based treatment. The recruited patients comprised 14 men and 8 women with a median age of 44.5 (range:24-76) years at relapse. 1 (5%) patient had microgranular variant of APL while 2 patients (9%) had therapy-related APL. Additional karyotypic abnormalities were seen in 3 (14%) patients. 14 patients (64%) had central nervous system (CNS) involvement at relapse. Internal tandem duplication of *FLT3* (*FLT3*-ITD) was detected in 7 (32%) patients. The following recurrent mutations were found: *NOTCH2* (n=17, 77%), *FOXD4L5* (n=22, 100%), *KCNJ11/18* (n=14, 64%), *MADCAM1* (n=15, 68%), *CBR3* (n=17, 77%), *NSD1* (n=17, 77%), *FLG1* (n=20, 91%), *CCDC179* (n=7, 32%), *SIGLEC11* (n=5, 23%), *ROBO4* (n=3, 14%), *CISD2* (n=3, 14%), *WT1* (n=1, 5%), *PTCH1*(n=1, 5%), *KMT2D* (n=1, 5%) and *MED17* (n=6, 27%).

CONCLUSIONS

Mechanisms other than *PML* B2 domain mutations may account for As₂O₃ resistance. Novel genes regulating the apoptotic pathways, cellular proliferation, histone modification, DNA repair and angiogenesis were frequently mutated in As₂O₃-refractory APL. Further functional validation on the role of these mutations in conferring As₂O₃ resistance is required.