Title	Whole exome sequencing identifies novel mutations in relapsed or refractory acute promyelocytic leukaemia failing treatment with oral arsenic trioxide
Author(s)	Singh, GHH; Ip, HW; Li, J; Yim, RLH; Panagiotou, G; Leung, AYH; Kwong, YL
Citation	The 21st Congress of the European Hematology Association (EHA 2016), Copenhagen, Denmark, 9-12 June 2016.
Issued Date	2016
URL	http://hdl.handle.net/10722/225706
Rights	This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

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

Harinder Gill[1], *Ho-Wan Ip*[2], *Jun Li*[3], *Rita Yim*[1], *Panagiotou Gianni*[3] *Anskar Y.H. Leung*[1], *Yok-Lam Kwong*[1]

1: Department of Medicine, the University of Hong Kong, Hong Kong SAR.

2: Department of Pathology, the University of Hong Kong, Hong Kong SAR.

3: School of Biological Sciences, the University of Hong Kong, Hong Kong SAR.

BACKGROUND

Arsenic trioxide (As_2O_3)-based regimens is effective in inducing a remission in more than 90 percent of relapsed acute promyelocytic leukaemia (APL). Treatment failure following As_2O_3 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 As_2O_3.

AIMS

To determine mutations associated with resistance to oral As_2O_3 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 As_2O_3. Paired BM samples (As_2O_3-resistant sample was paired with the corresponding As_2O_3-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 As_2O_3 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 As_2O_3-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 As_2O_3-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, CCCDC179, 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 As_2O_3-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%), *CCCDC179* (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 As_2O_3 resistance. Novel genes regulating the apoptotic pathways, cellular proliferation, histone modification, DNA repair and angiogenesis were frequently mutated in As_2O_3-refractory APL. Further functional validation on the role of these mutations in conferring As_2O_3 resistance is required.