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TITLE

GWAS for the Identification of Genetic Variants Predictive of Cytarabine in vitro Sensitivity in Pediatric AML

HYPOTHESIS:
To identify SNPs predictive of cytarabine in vitro chemosensitivity of leukemic cells obtained from pediatric acute myeloid leukemia (AML) patients (St Jude AML02 clinical trial) at diagnosis.

BACKGROUND/AIMS:
AML is the second most common pediatric leukemia with the worst prognosis of all major cancers in pediatrics. Resistance is the most common reason for failure and adverse side effects also contribute to morbidity and mortality. Since cytarabine is the mainstay of AML treatment, the wide interpatient variability in response to cytarabine complicates chemotherapy further. This necessitates the identification of additional genetic variants important in the susceptibility to cytarabine cytotoxicity in order to allow for personalized treatment that maximizes efficacy and minimizes toxicity. Previous studies have utilized genome-wide gene expression signatures to identify markers associated with cytarabine response. Few studies have also utilized lymphoblastoid cell lines from normal individuals available through the HapMap project to perform genome-wide association analysis (GWAS) to identify genetic variants and gene-expression associated with in vitro cellular cytotoxicity to cytarabine. However, to best of our knowledge no GWAS study has so far been performed on in vitro cytarabine chemosensitivity of leukemic cells obtained from pediatric AML patients.

METHODS:
Leukemic cells from diagnosis obtained from N=65 patients were treated with varying concentrations of cytarabine and IC50 values were obtained. Samples were divided into resistant (n= 15; IC50 = 5µg/µl) and sensitive groups (n=50; IC50 < 5µg/µl). Genome-wide SNP data were generated using Illumina Omni 2.5M and Exome Beadchip SNP array at University of Miami, Hussman Institute for Human Genomics. We performed the standard GWAS QC procedure in order to remove SNPs with call rate < 95%, monomorphic SNPs, SNPs with MAF < 5% and samples with call rate < 95%. Following QC, GWAS was performed on the remaining high quality SNPs (n=1,317,146) to identify if any of these are significantly associated with resistant or sensitive groups. Second, genes to which SNPs passing the suggestive threshold (p < 1x10^-4) map and genes within +/- 500kb of these SNPs were tested for differential expression between sensitive and resistant groups using T-test. Third, SNPs mapping to genes or SNPs within +/- 500kb from genes that were differentially expressed (p < 0.05) were then tested to identify those having an eQTL effect (p < 0.05) using T-test/ANOVA.

RESULTS & CONCLUSIONS

Although none of the SNPs reached genome-wide significance (p < 5.0 x 10^-8), 113 SNPs located within or near 69 unique genes reached the suggestive level (p < 3x10^-4). Among the top candidate SNPs were GPR56 rs1376041 G>A and rs75400242 G>A SNP found within 500kb of the IGF1R gene. For both these SNPs, the minor allele A was significantly associated with cytarabine resistance (p=1.42x10^-5 and p=2.19x10^-5, respectively). Furthermore, the minor allele of rs1376041 and rs75400242 was associated with a significantly higher level of GPR56 and IGF1R gene expression levels (p=0.0195 and p=0.0445, respectively). Consistent with this observation, for both GPR56 and IGF1R, higher leukemic cell gene expression was associated with resistant cases as compared to sensitive cases (p=0.0313 and p=0.0270, respectively).

In conclusion, these results suggest that these SNPs might contribute to cytarabine in vitro sensitivity through regulation of gene expression. This is biologically relevant since it has been documented that GPR56 expression is associated with cytarabine resistance and poor outcomes in AML. In fact, recent data suggests high expression of GPR56 to contribute to AML development and identified it as a potential target for antibody targeted therapy. For IGF1R, consistent with our results it has been associated with promoting growth of AML cells and cytarabine resistance as well. Future functional pharmacogenomic studies will help in better understanding the mechanisms behind the SNP associations reported here.