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Genomics of primary chemoresistance and remission induction failure in paediatric and adult acute myeloid leukaemia

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Authorship

FB and AK wrote the manuscript and performed analysis. FB, PC, ES performed laboratory experiments. JH, SZ, SB, DP and BY performed analysis. KK, TA, SM, RS, SK, GM, JB compiled patient samples. ED, MG and JH performed statistical analysis. AK and RL designed the study. RL contributed clinical and genomic expertise.

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Abstract

Cure rates of children and adults with acute myeloid leukaemia (AML) remain unsatisfactory partly due to chemotherapy resistance. We investigated the genetic basis of AML in 107 primary cases by sequencing 670 genes mutated in haematological malignancies. *SETBP1*, *ASXL1* and *RELN* mutations were significantly associated with primary chemoresistance. We identified genomic alterations not previously described in AML, together with distinct genes that were significantly overexpressed in therapy-resistant AML. Defined gene mutations were sufficient to explain primary induction failure in only a minority of cases. Thus, additional genetic or molecular mechanisms must cause primary chemoresistance in paediatric and adult AML.

Keywords

Primary chemoresistance; Paediatric leukaemia; Cytogenetically normal acute myeloid leukaemia; Targeted deep sequencing genomics; Induction failure

For patients with acute myeloid leukaemia (AML), failure to achieve complete remission after induction therapy or relapse after complete remission represents the major barrier to cure for both children and adults. Despite recent efforts to improve risk stratification and to incorporate targeted therapies into AML therapy, relapse rates remain approximately 30% and 50% for children and adults with AML, respectively (de Rooij et al., 2015, Breems et al., 2005). Whilst leukaemia cytogenetics at the time of diagnosis has proven to be the most useful prognostic biomarker of clinical outcomes and therapy stratification, patients with cytogenetically normal (CN) AML encompass the largest group for whom prognostication and therapy selection are hindered by the lack of prognostic markers and effective therapeutic targets.

Genomic profiling has been used to stratify patients with CN AML, identifying recurrent somatic gene mutations, such as those in *NPM1*, *TET2*, *ASXL1*, and *DNMT3A*, that are significantly associated with clinical outcomes (Kihara et al., 2014). Recent studies using whole-genome sequencing have assessed the mutational landscape of relapsed AML in adults, identifying two major patterns of clonal evolution in response to chemotherapy: 1) persistence of the dominant pre-leukaemic clone with mutations acquired at relapse and 2) expansion of a diagnostic sub-clones, emphasizing the importance of therapeutically targeting specific molecular mechanisms that contribute to chemotherapy resistance (Ding et al., 2012). Similar observations were recently made using genomic studies of paediatric AML, with responses of specific genetic clones representing potential prognostic indicators (Farrar et al., 2016). Clonal persistence after induction chemotherapy appears to be associated with the increased risk of relapse (Klco et al., 2015), but whether these mutant clones directly cause chemoresistance or reflect age-related clonal haematopoiesis remains to be determined (Wong et al., 2016). Importantly, the molecular basis of primary chemotherapy resistance, and thus fundamental causes of chemoresistance in general, remain poorly understood. Here, we used targeted gene sequencing of the majority of genes

known to be recurrently mutated in haematological malignancies to identify diagnostic gene mutations that are associated with primary chemoresistance in adult and paediatric CN AML.

Specimens were collected from patients treated at the Memorial Sloan Kettering Cancer Center, MD Anderson Cancer Center, Dana-Farber Cancer Institute, Ohio State University Comprehensive Cancer Center and Children's Oncology Group (COG) participating institutions. All patients provided informed consent and were enrolled on respective institutional research protocols. Primary chemoresistance was defined based on the presence of at least 5% of abnormal blasts by morphological and immunophenotypic assessment of bone marrow aspirates obtained after two cycles of induction chemotherapy, as assessed by respective institutional or central pathological reviews. Detailed description of methods of specimen preparation is provided in the Supplementary Methods.

Targeted DNA and RNA capture and sequencing of 670 genes (405 genes in DNA and 265 genes in RNA) known to be recurrently mutated in haematological malignancies were performed using the Foundation One (Cambridge, MA) Heme sequencing platform. High quality reads were obtained from Illumina sequencing at an average read depth of 503 x (range 263–746 x) and variants were annotated and filtered as previously reported (He et al., 2016). Variant allele frequency (VAF) was used to estimate mutation clonality (Supplementary methods). Differential gene expression analysis was performed based on per million processing regions (PPM) gene expression measurements, as median normalized to all other AML specimens analysed to date (He et al., 2016). Detailed statistical analyses are described in Supplementary methods.

In total, genomic profiles of 107 CN AML specimens were obtained from 55 adults and 52 children (Table I). No significant differences were observed in the total number of coding mutations between the primary failure of induction chemotherapy and complete remission outcome groups for both paediatric and adult cohorts (Table SI). Although the adult cohort represents a heterogeneous population based on varying treatment regimens, genetic status (both number of mutations per patient and specific alterations) was not significantly different between induction therapy programs (Table SI). Consistent with previous studies, the most frequently mutated genes among adults were *DNMT3A* (49%), *FLT3* (42%) and *NPM1* (42%), and for paediatric patients *FLT3* (54%), *NRAS* (27%) and *WT1* (25%), which were not significantly associated with either outcome group (Figure 1). *DNMT3A* and *NPM1* mutations were relatively rare in paediatric (2%) as compared to adult cases ($p < 0.001$, permutation test). In contrast, heterozygous *CEBPA* mutations were more frequently observed in paediatric as compared to adult CN AML (21% versus 2%, respectively, $p < 0.001$, permutation test). Genomic profiling also identified 15 gene rearrangements among the 21 specimens for which diagnostic material of adequate quality was available for RNA capture (Table SI). Cryptic rearrangements of known genes in AML were detected, including *KMT2A* (*MLL*) and *NUP98-NSD1*. The latter was observed uniquely in paediatric patients from this study, consistent with previous reports (Hollink et al., 2011). We identified several cryptic gene rearrangements, including intergenic deletions involving *NOTCH1/EDF1* and *GATA2/DNAJB8*, which have not been previously described, as well as translocations leading to the rearrangements of *SQSTM1-NUP214* and *IGH-BCL2*.

Mutations of these genes have been reported in haematological malignancies, e.g. *SQSTM1-NUP214* gene fusion in cases of T-cell acute lymphoblastic leukaemias (ALL), but not in AML (Gorello et al., 2010). In addition, our study identified potential novel coding mutations in *MAP3K1 (MEKK)*, *MAP3K6 (MEKK6)*, *EP300 (p300)*, and *WDR90* (Table SI). These findings emphasize the value of genomic profiling to identify potential pathogenic lesions that may elude conventional cytogenetic analyses and diagnosis based on morphological or immunophenotypic studies.

We assessed whether any gene mutations were significantly associated with primary chemoresistance as compared to complete remission among the adult and paediatric patients in the studied cohorts. This identified mutations of *SETBP1* (8 out of 50 versus 1 out of 57, $p = 0.01$) and *ASXL1* (7 out of 50 versus 1 out of 57, $p = 0.02$), as well as *RELN* (8 out of 50 versus 2 out of 57, $p = 0.04$, permutation test). With the exception of *ASXL1*, which exhibited equal numbers of clonal and subclonal mutations, all *SETBP1* and 70% of *RELN* mutations were clonal (Table SII). None of the other apparently subclonal mutations, including those of *FLT3*, *NPM1*, *KIT* and *NRAS*, were found to be significantly associated with primary chemoresistance, when adjusted for multiple hypothesis testing (Table SII). Mutations of *SETBP1* and *ASXL1* have been associated with inferior outcomes and increased risk of relapse in patients with AML (Meggendorfer et al., 2013). Relapse-associated *SETBP1* mutations in AML commonly cause missense mutations in the SKI domain (p.858–871). Our analysis identified the missense p.D868N *SETBP1* mutation in one CN AML specimen, as well as additional missense mutations of unknown significance (p.E183K, p.A374T, p.A408T, p.M502I, p.D1161H, p.T1547N and p.S1590C), suggesting that alternative mechanisms of *SETBP1* dysregulation may exist in AML. Mutations of *RELN* have been reported in early T-cell precursor (ETP) ALL, a stem cell-like leukaemia with high rates of intrinsic chemotherapy resistance and genetic features that are shared with subtypes of AML (Zhang et al., 2012). Our findings of *RELN* mutations associated with primary chemoresistance in CN AML raise the possibility that these leukaemias may comprise a distinct biological entity.

Using the available RNA sequencing and gene expression data on 21 primary AML specimens from this cohort (He et al., 2016), we assessed whether the genes included in our analysis were significantly altered in expression in the induction failure as compared to complete remission cohorts (Table SIII). This analysis revealed significant overexpression of genes encoding DNA damage repair and response regulators [*ATM*, *ATR*, *PSIP1 (LEDGF)*, and *EMSY (C11ORF30)*], an endoplasmic reticulum stress response regulator (*HERPUD1*), transcription factors (*CBFB*, *MEF2C*), kinases [*AKT3* and *MAP3K7 (TAK1)*], metabolic enzymes (*LDHA*, *ACSL6*), clathrin (*CLTC*) and an immune regulator (*CTLA4*; $p < 0.05$, t-test). Indeed, recent studies have implicated *MEF2C* overexpression with inferior outcomes in paediatric AML (Laszlo et al., 2015). Additional profiling and functional studies will be necessary to establish their prevalence and pathogenicity in AML. These results suggest that other mechanisms, such as those involving epigenetic or functional molecular alterations of cell signalling, may also mediate chemotherapy response and failure.

In summary, we have assessed the mutational landscape of CN AML at diagnosis in adults and children to determine the genetic basis of induction failure and primary

chemoresistance. In spite of careful selection of cytogenetically normal specimens, our study identified numerous cryptic gene rearrangements, including those that have potential therapeutic implications. Likewise, we found novel gene alterations including lesions that have been observed in non-myeloid haematological malignancies, some of which may be pathogenic. In addition, this analysis revealed significant overexpression of genes that are associated with primary chemotherapy resistance. Though mutations of *SETBP1*, *ASXL1* and *RELN* appear to be associated with primary chemoresistance and induction failure in our study, their prevalence was relatively low. We did not identify single gene mutations that appeared to be significantly associated with primary chemoresistance and induction failure in the majority of the patients studied. It is possible that additional genetic lesions, such as those not included in the current target gene panel (He et al., 2016), or alternatively, combinations of genetic alterations that are below the statistical power of our study may cause chemotherapy resistance and failure in AML, which may be elucidated by larger and more comprehensive genomic profiling studies. Finally, it remains to be determined whether functional molecular profiling, such as that achieved using gene expression, epigenetic and proteomic analyses, may reveal generally useful biomarkers and therapeutic targets to prospectively identify and therapeutically block chemotherapy resistance in AML (Chan et al., 2015, Rijal et al., 2015).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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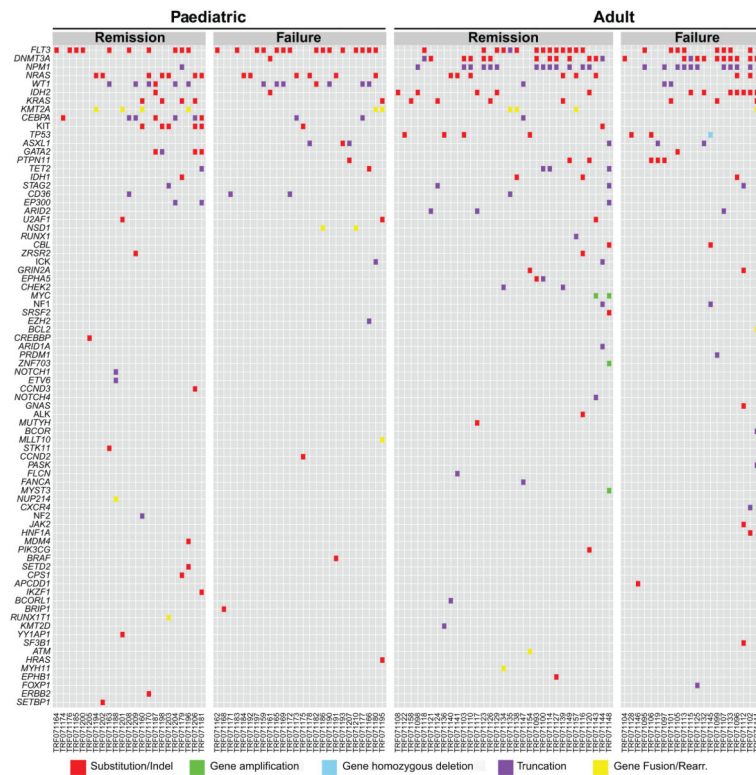


Figure 1. Mutational spectrum of paediatric and adult acute myeloid leukaemia with primary chemotherapy resistance and induction failure

Tile plot of the genomic alterations identified in DNA/RNA targeted sequencing. Data are shown for 107 cytogenetically normal acute myeloid leukaemia specimens, collected from 52 and 57 adult and paediatric specimens respectively, grouped by clinical outcome. Only genomic alterations of known pathological significance are displayed (Table SI).

Table I

Demographic features of induction failure and induction remission cohorts

Characteristics	Paediatric		Adult	
	Remission	Failure	Remission	Failure
Number, N (%)	25 (48)	27 (52)	32 (58)	23 (42)
Gender, % female	52	44	47	39
Age (years), median (range)	11.5 (2.8–16.8)	10.4 (0.6–16.8)	48.4 (19–84)	54.7 (16–73)
Therapy, N (%)				
ADE	25 (48)	27 (52)	0	0
IDA + HDAC	0	0	10 (31.2)	4 (17.4)
7+3 DNR and AraC	0	0	11 (34.4)	14 (60.9)
FAI	0	0	4 (12.5)	1 (4.3)
BID-FA	0	0	2 (6.25)	2 (8.7)
FLAG-IDA	0	0	4 (12.5)	0 (0)
IDA + AraC	0	0	1 (3.1)	1 (4.3)
Clofa + IDA + AraC	0	0	0	1 (4.3)

Abbreviations: ADE, cytarabine, daunomycin and etoposide-based regimen; IDA, idarubicin; HDAC, high-dose cytarabine; DNR, daunorubicin; BID-FA, twice daily fludarabine + cytarabine; FAI, fludarabine, cytarabine, idarubicin; FLAG-IDA, fludarabine, cytarabine, idarubicin and granulocyte colony stimulating factor; Clofa, clofarabine; AraC, cytarabine.