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Transcriptomic rationale for synthetic lethality-targeting *ERCC1* and *CDKN1A* in chronic myelomonocytic leukaemia

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Summary

Despite the absence of mutations in the DNA repair machinery in myeloid malignancies, the advent of high-throughput sequencing and discovery of splicing and epigenetics defects in chronic myelomonocytic leukaemia (CMML) prompted us to revisit a pathogenic role for genes involved in DNA damage response. We screened for misregulated DNA repair genes by enhanced RNA-sequencing on bone marrow from a discovery cohort of 27 CMML patients and 9 controls. We validated 4 differentially expressed candidates in CMML $CD34^+$ bone marrow selected cells and in an independent cohort of 74 CMML patients, mutationally contextualized by targeted sequencing, and assessed their transcriptional behavior in 70 myelodysplastic syndrome, 66 acute

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Authors' contributions

Contribution: A.M.H., D.H.W., F.FM. and A.J. were responsible for overall design, data collection, data analysis, data interpretation, statistical analysis, manuscript preparation, writing, and completion and final approval of manuscript; G.LG., T.H.CL., F.A., K.B., L.P., E.L., B.P., E.C., M.L.A., M.D-C. L.Z., E.S., J.M., F.J.O., V.V., C.dC. and F.S. gathered data and edited and approved the final manuscript.

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The authors have nothing to disclose.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article:

myeloid leukaemia and 25 chronic myeloid leukaemia cases. We found *BAP1* and *PARP1* down-regulation to be specific to CMML compared with other related disorders. Chromatin-regulator mutated cases showed decreased *BAP1* dosage. We validated a significant over-expression of the double strand break-fidelity genes *CDKN1A* and *ERCC1*, independent of promoter methylation and associated with chemorefractoriness. In addition, patients bearing mutations in the splicing component *SRSF2* displayed numerous aberrant splicing events in DNA repair genes, with a quantitative predominance in the single strand break pathway. Our results highlight potential targets in this disease, which currently has few therapeutic options.

Keywords

poly ADP-ribose polymerase 1 (*PARP1*); excision repair cross-complementation group 1 (*ERCC1*); cyclin dependent kinase inhibitor 1A (*CDKN1A*); CMML transcriptome; synthetic lethality

Defective DNA repair is a common hallmark of cancer. Mutations in genes encoding core components of DNA repair pathways are frequent across many malignancies, mostly solid tumors (Ciriello et al, 2013). Chronic myelomonocytic leukaemia (CMML) is characterized by a persistent blood monocytosis and overlapping pathological features of both myeloproliferative neoplasm and myelodysplastic syndrome (MDS) (Swerdlow et al, 2008). Compared to MDS and acute myeloid leukaemia (AML), losses of heterozygosity due to cytogenetically-detectable chromosomal deletions or gains are uncommon in CMML, whereas point mutations are more frequent, suggesting different underlying defects generating genomic errors (Such et al, 2011; Jerez et al, 2012; Kar et al, 2013).

Neoplastic cells with particular DNA repair lesions can instead fully rely on alternative repair pathways for their survival. This dependence can be targeted to induce synthetic lethality in malignant cells (Bryant et al, 2005; Lee et al, 2012). The lack of recurrent, pathogenic somatic mutations in DNA repair mechanism components in myeloid malignancy has diverted attention away from interest in this pathway (Yoshida et al, 2011; Huang et al, 2015; Papaemmanuil et al, 2016). However, recent studies have revealed the need for a fine-tuned equilibrium in stimulating DNA repair machinery that can prevent mutations, but which can also preclude the extinction of malignant blood cells by therapeutic agents (Tong et al, 2016). Splicing factor and microsatellite anomalies disrupting genomic integrity maintenance and the biological rationale for combining polymerase inhibitors and demethylating agents are both recent findings that cause convergence between myeloid cancer and DNA repair (Gaymes et al, 2013; Dolatshad et al, 2015; Muvarak et al, 2016; Pederiva et al, 2016).

The main goals of this study were: (i) to screen, by means of global and massive sequencing, for anomalies in the transcriptome of genes involved in the DNA repair machinery in patients with CMML, identifying targetable candidates; (ii) to validate their misregulation in an independent large CMML cohort, establishing their genomic and clinical context; and (iii) to assess potential neoplasm-specificity by determining how those candidates behave through the spectrum of myeloid malignancies.

Material and methods

Patients and samples

Bone marrow (BM) aspirates were collected during the diagnostic workup from 259 patients presenting to University Hospitals from Salamanca and Badalona (27 CMML discovery cohort cases) and to University Hospitals from Murcia [validation cohort of 74 CMML cases; and 70 MDS, 53 AML and 25 chronic myeloid leukaemia (CML) patients]. Diagnoses were made according to 2008 and 2016 World Health Organization classification (Vardiman et al, 2009; Arber et al, 2016). In each case, informed consent was obtained in accordance with protocols approved by each centre's Institutional Review Board and with the Declaration of Helsinki.

RNA-sequencing (RNA-Seq)

RNA extracted from BM samples obtained from 27 CMML patients and 9 healthy controls was used for deep RNA-Seq (mean 106 million reads per sample) on a HiSeq4000 sequencer (Illumina, San Diego, CA, USA). In five patients treated with azacitidine, a second BM sample at day +28 after 3 cycles of azacitidine was also sequenced. We used LIMMA package or empirical analysis of digital gene expression data in R (edgeR) (both available at: www.bioconductor.org) to identify differential expression. Alternative 3′ and 5′ splice sites, skipped exons, mutually exclusive exons and retained introns were quantified using rMATS with the assembly produced from STAR (both available at: http://rnaseq-mats.sourceforge.net/) (Shen et al, 2014). The default parameters were used for the comparison of the samples.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR), $CD34^+$ cells isolation, and flow cytometry

Differentially expressed genes (DEGs) results were validated by using TaqMan gene expression assays with probes from Thermo Fisher Scientific, Waltham, MA (Table SI). $CD34^+$ cells were enriched from thawed BM using magnetic microbeads and an autoMACS® Pro Separator (Miltenyi Biotec, Bergisch-Gladbach, Germany). The granulomonocytic compartment was quantified in the discovery cohort using CD11b-fluorescein isothicyanate (FITC), CD13-phycoerythrin (PE), CD45− peridinin-chlorophyll (PerCP), and CD34− allophycocyanin (APC) performed on a FACSCanto II (Becton-Dickinson, San Jose, CA, USA).

DNA methylation analysis by pyrosequencing

Primer sequences are listed in Table SII. Bisulphite conversion of 500 ng of each DNA sample was performed with the EZ DNA Methylation-Gold Kit (Zymo Research, Milan Italy).

DNA targeted sequencing

We designed a TruSeq Custom Amplicon panel (Illumina, Inc. San Diego, CA, USA) including 18 genes recurrently mutated in CMML (Table SIII). Sequencing was performed with MiSeq v2.2 chemistry, and a mean depth of 982 reads/base was obtained.

Statistics

Analysis was performed using Student-*t* and Mann–Whitney-*U* test. The Kaplan–Meier method was used for survival outcomes. Significance was determined at a two-sided alpha level of 0·05, except for *P* values in multiple comparisons, for which Bonferroni correction was applied (SPSS version 21.0; IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA).

Additional details on control samples, techniques and open databases are provided in Data S1.

Results

Global DNA repair genes expression in CMML: RNA-Seq

To investigate detailed gene expression signatures for the genes involved in DNA repair pathways we performed RNA-Seq on bone marrow in a discovery cohort of 27 CMML cases and 9 healthy controls. Donors (mean, 65 years of age.) were selected to match the advanced age of CMML patients. Main baseline patients' characteristics depicted in Table I. Of the 204 genes considered in this analysis, the expression of 30 genes was significantly different between the two groups, with 8 genes up-regulated and 22 genes down-regulated in CMML patients (Figure S1, upper panel, and Table SIV).

Defects in genes predominantly unique to a single strand break (SSB) repair pathway included: *NEIL1* and *OGG1* in base excision repair (BER), *XPA* and *MMS19* in nucleotide excision repair (NER) and *RPA4* in mismatch repair (MMR). *XRCC4* and *MSH4* overexpression and *PRKDC* down-regulation were significant changes among genes associated exclusively with double strand break (DSB) repair. Differences in genes coding for multifunctional proteins, involved in both SSB and DSB, and/or common damage checkpoints were also observed. These included down-regulation of *PARP1, PNKP, LIG1, POLE, MSH2, MDC1, FANCD2, BLM, BAP1, POLH* and *MUS81*; and up-regulation of *ERCC1, TDP1* and *POLK*.

We next investigated whether genes exclusively involved in DNA damage checkpoints were altered in CMML patients. We found relative underexpression of two SSB sensors, *ATRIP* and *RAD9A*; two common mediators/effectors, *MDC1* and *CDC25B*; and one cell cycle promoter, *CDK2*. Two potent cyclin-dependent kinase inhibitors, *CDKN1A* (otherwise known as *p21*) and *CDKN1C*, demonstrated 5- and 2-fold increased expression, respectively, compared with healthy individuals.

Targetable DNA repair differentially expressed genes: validation and behaviour through the myeloid spectrum

We selected four of the 30 RNA-Seq DEGs for validation in CMML $CD34^+$ BM selected cells, in an independent CMML cohort and further characterization through the spectrum of myeloid neoplasms. Candidate genes for validation were selected on clinical grounds, specifically targeting those predicted to have the highest translational potential. Categories selected were: (i) druggable oncogenes found to be highly overexpressed, allowing

for modulation of a specific DNA repair pathway (*CDKN1A, ERCC1*); (ii) oncogenes underexpressed but with inhibitory molecules already being tested in cancer (*PARP1*), (Zereshkian et al, 2014; Muvarak et al, 2016) and (iii) genes down-regulated and with a previously documented association with CMML pathogenesis (*BAP1*) (Dey et al, 2012). The CMML validation cohort included 74 patients (Table I). Gene expression levels for the 4 validation genes were quantified by RT-qPCR and compared with expression data from 9 healthy bone marrow donors.

Corroborating the findings from our discovery cohort, we observed statistically significant and direction-concordant dysregulation for all four of the shortlisted genes (*CDKN1A, ERCC1, PARP1, BAP1*) in the independent RT-qPCR CMML validation cohort (Fig 1 and Table SV): *CDKN1A* showed a 3 log fold change (logFC) overexpression compared with controls ($P = 0{\cdot}01$), and we validated *ERCC1* to be up-regulated in patients (1·8 logFC *versus* controls, $P < 0{\cdot}001$); *PARP1* and *BAP1* were confirmed to be down-regulated when comparing the validation CMML cohort *versus* controls (*PARP1* −0·5 logFC, $P = 0{\cdot}047$); *BAP1* −0·6 logFC, $P = 0{\cdot}008$).

It could be hypothesized that the distinct expression in CMML BM cells of our candidate genes could be a feature that distinguishes the monocytic compartment, either healthy or leukaemic, and that the differences found herein are due to the higher proportion of monocytic cells in this disease. To test that hypothesis, we measured, by flow cytometry, the myeloid, monocytic, $CD34^+$ and lymphoid subset in 18 unfractionated bone marrow samples from the discovery cohort. The median percentage represented by the granulomonocytic compartment (82%), comprised a median of 59% granulocytes, 22% monocytes and 08% myeloid $CD34^+$ cells. No correlation was found between the size of those compartments and the expression of *CDKNA1, PARP1, ERCC1* or *BAP1* (Table SVI).

The RT-qPCR expression analysis for *PARP1, CDKN1A, ERCC1* and *BAP1* was then extended to our own series of MDS ($n = 70$), AML ($n = 53$) and CML ($n = 25$) patients (Fig 1 and Table SV). Similar to CMML, *ERCC1* was uniformly up-regulated in every entity tested, whilst *CDKN1A* was markedly overexpressed in each disorder with the exception of CML. Interestingly, *PARP1* and *BAP1* displayed opposite directions of dysregulation in CMML and CML cases (underexpressed) *versus* MDS and AML (overexpressed). Microarray Innovations in LEukaemia MILE study microarray data reproduced the direction of misregulation of *PARP1, CDKN1A, ERCC1* and *BAP1* in every disease subset tested (Haferlach et al, 2010).

$CD34^+$ cells PARP1, CDKN1A, ERCC1 and BAP1 in CMML, MDS and donors

Total RNA was extracted from $CD34^+$ BM cells selected (with a median average purity of 95%) from 15 CMML patients (from the RNA-Seq discovery cohort), 15 MDS cases and 15 healthy donors, and studied by RT-qPCR (Figure S2). Compared with BM $CD34^+$ from controls, we confirmed the upregulation of *CDKN1A* in CMML $CD34^+$ (1·5 logFC, $P = 0{\cdot}03$), *ERCC1* (0·3 logFC, $P = 0{\cdot}04$) and the downregulation of *PARP1*(−0·6 logFC, $P = 0{\cdot}02$) and *BAP1* (−0·3 logFC, $P = 0{\cdot}04$).

Misregulated expression of DNA repair genes in CMML is not mediated by aberrant promoter CpG methylation

We hypothesised that differential promoter CpG methylation patterns might explain the observed differences in expression for the CMML DEGs. To explore this we quantified the DNA methylation status of 48 CpG sites, located within the proximal promoter regions of 26 out of the 30 RNA-Seq DEGs, from 22 CMML samples and 4 healthy donors obtained using the Human Methylation27 Beadchip (Table SVII) (Pérez et al, 2012). We considered a gene locus to be unmethylated when its beta value was lower than 0·2. Strikingly, with the exception of hypermethylation observed at *MSH4*, every CpG on every other DNA repair gene tested was unmethylated.

Pyrosequencing was used to confirm the unmethylated status of our main candidates for a synthetic lethality approach: the overexpressed double-break fidelity genes *ERCC1* and *CDKN1A*. Lack of methylation of their promoter CpGs would add rationale for incorporating a different modulation if these genes to currently used hypomethylating agents. Our design covered two regions in the *CDKN1A* promoter (including 5 CpGs) and one region in the *ERCC1* promoter (including 3 CpGs). In agreement with the microarray data, all CpGs probed by this method were found to be unmethylated, both in bone marrow samples from 56 CMML cases and 16 controls (Table SVIII).

Therefore, promoter methylation does not appear to be a mechanism by which DNA repair genes are differentially expressed in CMML *versus* healthy BM. Consistent with this, patients treated with the hypomethylating agent azacitidine for 3 cycles failed to show any significant correction of the aberrant expression across any of the identified DNA Repair DEGs (Fig 2). This was the case for both clinical responders ($n = 2$) and non-responders ($n = 3$).

Mutational correlates for CMML DNA Repair DEGs

We next investigated whether DNA Repair DEGs in CMML were associated with particular patterns of recurrent mutations. We perfomed targeted amplicon sequencing using a panel of 18 genes known to be recurrently mutated in CMML in 57 samples with available DNA from the CMML validation cohort. A total of 147 non-synonymous somatic variants were identified (Figure S3 and Table SIX), including typical CMML-associated mutations and frequencies: *TET2* (total number of mutations = 82, 77% cases mutated), *SRSF2* ($n = 15$, 26%), and *ASXL1* ($n = 9$, 16%),

Next, we compared expression of the shortlisted CMML DEGs, *CDKN1A, ERCC1, PARP1* and *BAP1*, across patient groups displaying mutations in *TET2*, in a splicing factor (*SRSF2, SF3B1, ZRSR2*), or in a chromatin regulator (*ASXL1, EZH2*). We found significantly decreased expression of *PARP1* in *TET2*-mutated cases (−0·69 logFC, $P = 0·02$), and of both *CDKN1A* (−2·5 logFC, $P = 0·01$) and *BAP1* (−0·2 logFC, $P = 0·04$) in chromatin regulator-mutated cases. Presence of a splicing factor mutation was not associated with specific expression patterns for the observed CMML DNA repair DEGs (data not shown).

SRSF2 mutations induce widespread aberrant splicing events in DNA repair genes in CMML

Given that mutations in specific spliceosome components have distinct impacts on splicing and that *SRSF2* mutations are by far the most common such mutations in CMML, (Meggendorfer et al, 2012) we focused our analysis on CMML patients harbouring typical *SRSF2* proline 95 mutations ($n = 7$). Compared with patients with no mutations in any of the spliceosomal genes included in our panel ($n = 13$), at least one significant aberrant splicing event (inclusion ≥0·05; false discovery rate <0·05) was observed for 48 of the 204 DNA repair genes considered in this study (Fig 3 and Tables SX–XIV). Exon skipping/cassette exon events were most common, occurring in 42 genes; many of these displayed events involving multiple exons. Other significant events included mutually exclusive exons ($n = 16$ genes), alternative 3′ splice site ($n = 8$), alternative 5′ splice site ($n = 1$) and retained intron ($n = 1$).

Only a small minority of aberrant splicing events occurred in CMML DEGs: skipped exons in *MDC1, POLK* and *TDP1*; mutually exclusive exons in *MDC1* and *POLK*; and alternative 3′ splice sites in *POLH* and *XRCC4*. Thus, significant aberrant splicing events with potential to influence downstream function were found in an additional 43 DNA repair genes, beyond those demonstrating misregulated expression levels in CMML.

Clinico-biological correlates for CMML DNA Repair DEGs

Next, we investigated associations of the DNA repair DEGs with various clinical parameters in our validation cohort of 74 CMML patients (Table SXV). To this regard, cases with *CDKN1A* overexpression were characterized by fewer cytogenetic anomalies ($P = 0·033$).

It was previously described that *PARP1* is required for the generation of chromosomal translocations (Wray et al, 2013). As expected from its down-regulation, only one of 101 CMML patients across our discovery and validation cohorts presented with such a balanced translocation; this case was atypical in our cohort in displaying up-regulation of *PARP1* compared to controls. Extending analysis to our MDS and AML patients, *PARP1* expression was significantly higher in patients with ($n = 14$) *versus* without ($n = 97$) a translocation ($P = 0·03$; Tables SXVI and SXVII). AML patients who failed to achieve complete remission (CR) after induction chemotherapy displayed markedly higher expression of *CDKNA1* (22-fold change *versus* 11-fold change; $P = 0·02$) and *ERCC1* (25-fold change *versus* 14-fold change; $P = 0·03$), compared with those who successfully achieved CR.

Further insight into DNA repair transcriptome among myeloid disorders: comparison with the MILE study

We were interested to compare how the transcriptional changes identified in our CMML cohort compared with those in related myeloid disorders. We therefore compared our unbiased RNA-Seq data with results extracted from the MILE study (Mills et al, 2009; Haferlach et al, 2010), which compared gene expression by microarray on unfractionated bone marrow from patients with a variety of leukaemias *versus* healthy donors. Accordingly, transcriptional data for DNA repair components were extracted from a large dataset of 206 MDS, 351 normal karyotype AML (nkAML) and 48 complex karyotype AML (ckAML;

defined by the occurrence of at least three clonal chromosomal abnormalities) patients, and compared gene expression patterns *versus* 73 healthy donors.

Surprisingly, MDS samples from the MILE study demonstrated predominant up-regulation of DNA repair genes, with 14 of 20 misregulated targets up-regulated *versus* healthy donors (Figure S4 and Table SXVIII). By contrast, ckAML showed a global defect with predominance of down-regulated DNA repair components (37 of 50 misregulated targets) (Fig 1 lower panel and Table SXIX).

Overlay of significant expression patterns from our CMML dataset with MILE cohorts of MDS and ckAML patients revealed three shared genes misregulated in the same direction across all three cohorts: overexpression of *ERCC1*, and down-regulation of *NEIL1* and *CDC25B* (Figure S5).

Of note, several genes displayed antithetic misregulation depending on the underlying disease: *BAP1* was up-regulated in ckAML and down-regulated in CMML; whereas *CDK1* and *EXO1* were up-regulated in MDS with the opposite effect observed among ckAML cases. These findings suggest intriguing differences in pathogenic role for DNA repair transcriptional defects between these related but distinct clinicopathological entities.

Discussion

Differences in chromosomal and gene-level DNA lesions across the spectrum of myeloid neoplasms can result from diversity of the underlying DNA repair defects, pointing toward the existence of disorder-specific targets in this machinery. In this study, we identified distinct targetable and misregulated DNA repair genes in a deep RNA-Sequenced discovery cohort of CMML patients. Notably, the broad pattern differs from that extracted from previously published MDS and AML cases. We validated our identified candidates in BM $CD34^+$ CMML cells, in a large and independent series of CMML patients, and compared their behaviour through different myeloid disorders by means of a direct RT-qPCR technique. Beyond simple transcriptional expression changes, we observed a host of additional novel splicing abnormalities in CMML patients bearing mutations in the spliceosome component *SRSF2*.

Independently from its cell cycle checkpoint function, *CDKN1A* has been shown to regulate the accuracy of replication-coupled DSB repair and the maintenance of chromosome stability (Patel et al, 2017). Our results indicate a striking up-regulation of this gene in CMML patients, although not specific to this entity given that it is replicated across the spectrum of myeloid neoplasms. This overexpression did not correlate with presence of disrupted *TP53*, the main *CDKN1A* mediator, (Brugarolas et al, 1995) either by mutation or misregulation. Thus, targeting this DSB fidelity gene by its TP53-independent activity in cells with a relatively preserved DSB repair pathway emerges as an exciting and plausible synthetic lethality approach in CMML and other myeloid cancers.

ERCC1, initially placed among the NER components, has ubiquitous roles in the DNA damage response. Its defects produced unexpected deleterious and complex DNA lesions (McWhir et al, 1993). Subsequently, an increased sensitivity to interstrand cross link

(ICL)-inducing agents, and a key role in deleting non-homologous tails at rupture bounds before they are rejoined, established a predominant role in the DSB repair machinery (Kim et al, 2011; Ahmad et al, 2008). In our study, *ERCC1* gene dosage was significantly increased in CMML and MDS patients compared with controls, and to a still greater degree (doubled) in AML. Interestingly, as for *CDKN1A*, overexpression of *ERCC1* in AML patients was significantly associated with failure to achieve complete remission after induction chemotherapy.

Focal DNA hypermethylation in the context of broad hypomethylation is a hallmark of myeloid neoplasms, but this dysregulation is not homogeneous through the ambit of these diseases (Figueroa et al, 2010; Itzykson & Fenaux, 2014). In our CMML cohort, with the exception of *MSH4*, all promoter CpGs for all shortlisted DEGs extracted showed un unmethylated status. Moreover, we noted no significant reversal of gene expression changes after 3 cycles of azacitidine in a limited series of CMML patients, irrespective of their clinical response to treatment. This finding supports that DEGs in the DNA repair pathway are not directly subordinated to this epigenetic control mechanism and recasts their modulation as a suitable candidate for combination therapy alongside hypomethylating agents. Indeed, we directly confirmed in our cohort the demethylated status of *ERCC1* and *CDKN1A* promoters.

It was recently shown that alternative NHEJ (aNHEJ) elements are required for formation of chromosomal translocations (Zhang & Jasin, 2011). *PARP1*, the initiator of aNHEJ, is a key player in the molecular mechanism underlying this process, and its inhibition or repression markedly decreases or completely abrogates translocations, respectively (Wray et al, 2013). Our findings fully support these basic observations: the direction of *PARP1* misregulation was inverted in MDS and AML patients (upregulated; translocations present in a significant proportion of cases), compared with our CMML cohort (downregulated; translocations absent). The disease-specific differences in direction of misregulation may partially explain the different distributions of genomic lesions observed, but also underpins the need for neoplasm-personalized therapeutic modulation. Recent work has founded an *in vitro* biological rationale for combining *PARP1* and demethylating agents in myeloid disease (Muvarak et al, 2016). Our study indicates that responses may be heterogeneous across the myeloid spectrum, potentially less effective in CMML than described in MDS and AML models (Gaymes et al, 2009).

We detected and validated a significant underexpression of *BAP1* in CMML samples, shared only by CML patients among the myeloid diseases studied. This deubiquinase is mutated in various hereditary cancers and its deletion is associated with the appearance of myelodysplastic/myeloproliferative features in mice (Dey et al, 2012). *BAP1* function is intimately linked with that of *ASXL1*, commonly mutated in CMML, and it was recently shown that the interaction between *ASXL1* and *BAP1* was restored in *ASXL1*-mutation corrected clones (Valletta et al, 2015). Accordingly, our *ASXL1*-mutated CMML cases were characterized by a reduced *BAP1* gene dosage, that reached statistical significance when combined with cases bearing mutations in *EZH2*, another chromatin modulator. In addition, the reduction of *BAP1* transcripts in CML patients we identified replicates findings recently reported (Dkhissi et al, 2015). Although the granulomonocytic compartment represented a

median average of 82% of cells in the unfractionated bone marrow samples used in our CMML RNA-seq discovery cohort, it could be argued the abnormal repartition of cell lineages could be responsible of the changes detected. We show here that the alterations detected in the RNA-seq experiment were also validated in bone marrow $CD34^+$ cells from CMML and healthy donors.

Mutations in splicing factors are common within myeloid malignancies, but their role in pathogenesis has not been extensively delineated (Yoshida et al, 2011). Recent data show how mutant *SRSF2*, frequently mutated in CMML, promotes mis-splicing and degradation of *EZH2* in mice and cell lines (Kim et al, 2015). When comparing *SRSF2* mutated *versus* non mutated cases, only a minority of aberrant splicing events occurred in CMML DEGs, none in *ERCC1, CDKN1A, PARP1* and *BAP1*. Regarding non DEGs, three components of the SSB pathways, *EXO1, ENDOV* and *ERCC6* showed the highest rate of exon skipping (beyond −0·3) in mutant cases, pointing out the preference for defects in single strand repair mechanisms in CMML.

We uncovered different patterns of DNA repair gene misregulation when comparing our CMML cohort with the MDS and ckAML subsets of the MILE study. Perhaps unsurprisingly, a global disruption to all DNA repair pathways was observed in ckAML, with a remarkably damaged homologous recombination pathway. Conversely, we did not expect upregulation to be a hallmark of MDS, the prototypical “hypermethylation” disease.

In summary, we have identified, by means of an unbiased high-throughput approach, and validated in BM $CD34^+$ CMML cells and in an independent cohort, a subset of DNA repair genes consistently misregulated in CMML. We highlight meaningful clinical and mutational correlates, alongside relationships that point towards their compensatory nature within a damaged cancer DNA repair machinery. The genes identified warrant further study as potential novel therapeutic targets, both directly and through modulation of associated compensatory pathways. In particular, *CDKN1A* and *ERCC1* emerge as realistic candidates for a synthetic lethality approach. Indeed, we further identified important disease-specific differences for DNA repair DEGs comparing different myeloid malignancies, with potential translational ramifications for the positioning of novel therapeutic strategies. For example, our findings suggest that the promise of *PARP1* inhibition appears less pertinent to CMML than for other myeloid diseases. These differences might also partially explain the different genomic and phenotypic manifestations of different myeloid neoplasms and shed insights into their distinctive biology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Ahmad A, Robinson AR, Duensing A, van Drunen E, Beverloo HB, Weisberg DB, Hasty P, Hoeijmakers JHJ & Niedernhofer LJ (2008) ERCC1-XPF endonuclease facilitates DNA double-strand break repair. Molecular and Cellular Biology, 28, 5082–5092. [PubMed: 18541667]

Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M. & Vardiman JW (2016) The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood, 127, 2391–2405. [PubMed: 27069254]

Brugarolas J, Chandrasekaran C, Gordon JI, Beach D, Jacks T. & Hannon GJ (1995) Radiation-induced cell cycle arrest compromised by p21 deficiency. Nature, 377, 552–557. [PubMed: 7566157]

Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ & Helleday T. (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature, 434, 913–917. [PubMed: 15829966]

Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N. & Sander C. (2013) Emerging landscape of oncogenic signatures across human cancers. Nature Genetics, 45, 1127–1133. [PubMed: 24071851]

Dey A, Seshasayee D, Noubade R, French DM, Liu J, Chaurushiya MS, Kirkpatrick DS, Pham VC, Lill JR, Bakalarski CE, Wu J, Phu L, Katavolos P, LaFave LM, Abdel-Wahab O, Modrusan Z, Seshagiri S, Dong K, Lin Z, Balazs M, Newton K, Hymowitz S, Garcia-Manero G, Martin F, Levine RL & Dixit VM (2012) Loss of the tumor suppressor BAP1 causes myeloid transformation. Science, 337, 1541–1546. [PubMed: 22878500]

Dkhissi F, Aggoune D, Pontis J, Sorel N, Piccirilli N, LeCorf A, Guilhot F, Chomel JC, Ait-Si-Ali S. & Turhan AG (2015) The downregulation of BAP1 expression by BCR-ABL reduces the stability of BRCA1 in chronic myeloid leukemia. Experimental Hematology, 43, 775–780. [PubMed: 26118501]

Dolatshad H, Pellagatti A, Fernandez-Mercado M, Yip BH, Malcovati L, Attwood M, Przychodzen B, Sahgal N, Kanapin AA, Lockstone H, Scifo L, Vandenberghe P, Papaemmanuil E, Smith CWJ, Campbell PJ, Ogawa S, Maciejewski JP, Cazzola M, Savage KI & Boultwood J. (2015) Disruption of SF3B1 results in deregulated expression and splicing of key genes and pathways in myelodysplastic syndrome hematopoietic stem and progenitor cells. Leukemia, 29, 1092–1103. [PubMed: 25428262]

Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, Christos PJ, Schifano E, Booth J, van Putten W, Skrabanek L, Campagne F, Mazumdar M, Greally JM, Valk PJM, Löwenberg B, Delwel R. & Melnick A. (2010) DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. Cancer Cell, 17, 13–27. [PubMed: 20060365]

Gaymes TJ, Shall S, MacPherson LJ, Twine NA, Lea NC, Farzaneh F. & Mufti GJ (2009) Inhibitors of poly ADP-ribose polymerase (PARP) induce apoptosis of myeloid leukemic cells: potential for therapy of myeloid leukemia and myelodysplastic syndromes. Haematologica, 94, 638–646. [PubMed: 19407318]

Gaymes TJ, Mohamedali AM, Patterson M, Matto N, Smith A, Kulasekararaj A, Chelliah R, Curtin N, Farzaneh F, Shall S. & Mufti GJ (2013) Microsatellite instability induced mutations in DNA repair genes CtIP and MRE11 confer hypersensitivity to poly (ADP-ribose) polymerase inhibitors in myeloid malignancies. Haematologica, 98, 1397–1406. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23349304 (Accessed November 15, 2017). [PubMed: 23349304]

Haferlach T, Kohlmann A, Wieczorek L, Basso G, Te Kronnie G, Béné MC, De Vos J, Hernández JM, Hofmann WK, Mills KI, Gilkes A, Chiaretti S, Shurtleff SA, Kipps TJ, Rassenti LZ, Yeoh AE, Papenhausen PR, Liu WM, Williams PM & Foà R. (2010) Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: Report from the international microarray innovations in leukemia study group. Journal of Clinical Oncology, 28, 2529–2537. [PubMed: 20406941]

Huang D, Nagata Y, Grossmann V, Radivoyevitch T, Okuno Y, Nagae G, Hosono N, Schnittger S, Sanada M, Przychodzen B, Kon A, Polprasert C, Shen W, Clemente MJ, Phillips JG, Alpermann T, Yoshida K, Nadarajah N, Sekeres MA, Oakley K, Nguyen N, Shiraishi Y, Shiozawa Y, Chiba K, Tanaka H, Koeffler HP, Klein HU, Dugas M, Aburatani H, Miyano S, Haferlach C, Kern W, Haferlach T, Du Y, Ogawa S. & Makishima H. (2015) BRCC3 mutations in myeloid neoplasms. Haematologica, 100, 1051–1057. [PubMed: 26001790]

Itzykson R. & Fenaux P. (2014) Epigenetics of myelodysplastic syndromes. Leukemia, 28, 497–506. [PubMed: 24247656]

Jerez A, Sugimoto Y, Makishima H, Verma A, Jankowska AM, Przychodzen B, Visconte V, Tiu RV, O'Keefe CL, Mohamedali AM, Kulasekararaj AG, Pellagatti A, McGraw K, Muramatsu H, Moliterno AR, Sekeres MA, McDevitt MA, Kojima S, List A, Boultwood J, Mufti GJ & Maciejewski JP (2012) Loss of heterozygosity in 7q myeloid disorders: clinical associations and genomic pathogenesis. Blood, 119, 6109–6117. [PubMed: 22553315]

Kar SA, Jankowska A, Makishima H, Visconte V, Jerez A, Sugimoto Y, Muramatsu H, Traina F, Afable M, Guinta K, Tiu RV, Przychodzen B, Sakaguchi H, Kojima S, Sekeres MA, List AF, McDevitt MA & Maciejewski JP (2013) Spliceosomal gene mutations are frequent events in the diverse mutational spectrum of chronic myelomonocytic leukemia but largely absent in juvenile myelomonocytic leukemia. Haematologica, 98, 107–113. [PubMed: 22773603]

Kim Y, Lach FP, Desetty R, Hanenberg H, Auerbach AD & Smogorzewska A. (2011) Mutations of the SLX4 gene in Fanconi anemia. Nature Genetics, 43, 142–146. [PubMed: 21240275]

Kim E, Ilagan JO, Liang Y, Daubner GM, Lee SCW, Ramakrishnan A, Li Y, Chung YR, Micol JB, Murphy ME, Cho H, Kim MK, Zebari AS, Aumann S, Park CY, Buonamici S, Smith PG, Deeg HJ, Lobry C, Aifantis I, Modis Y, Allain FH, Halene S, Bradley RK & Abdel-Wahab O. (2015) SRSF2 mutations contribute to myelodysplasia by mutant-specific effects on exon recognition. Cancer Cell, 27, 617–630. [PubMed: 25965569]

Lee MJ, Ye AS, Gardino AK, Heijink AM, Sorger PK, MacBeath G. & Yaffe MB (2012) Sequential application of anticancer drugs enhances cell death by rewiring apoptotic signaling networks. Cell, 149, 780–794. [PubMed: 22579283]

McWhir J, Selfridge J, Harrison DJ, Squires S. & Melton DW (1993) Mice with DNA repair gene (ERCC-1) deficiency have elevated levels of p53, liver nuclear abnormalities and die before weaning. Nature Genetics, 5, 217–224. [PubMed: 8275084]

Meggendorfer M, Roller A, Haferlach T, Eder C, Dicker F, Grossmann V, Kohlmann A, Alpermann T, Yoshida K, Ogawa S, Koeffler HP, Kern W, Haferlach C. & Schnittger S. (2012) SRSF2 mutations in 275 cases with chronic myelomonocytic leukemia (CMML). Blood, 120, 3080–3088. [PubMed: 22919025]

Mills KI, Kohlmann A, Williams PM, Wieczorek L, Liu WM, Li R, Wei W, Bowen DT, Loeffler H, Hernandez JM, Hofmann WK & Haferlach T. (2009) Microarray-based classifiers and prognosis models identify subgroups with distinct clinical outcomes and high risk of AML transformation of myelodysplastic syndrome. Blood, 114, 1063–1072. [PubMed: 19443663]

Muvarak NE, Chowdhury K, Xia L, Robert C, Choi EY, Cai Y, Bellani M, Zou Y, Singh ZN, Duong VH, Rutherford T, Nagaria P, Bentzen SM, Seidman MM, Baer MR, Lapidus RG, Baylin SB & Rassool FV (2016) Enhancing the cytotoxic effects of PARP inhibitors with DNA demethylating agents – a potential therapy for cancer. Cancer Cell, 30, 637–650. [PubMed: 27728808]

Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, Potter NE, Heuser M, Thol F, Bolli N, Gundem G, Van Loo P, Martincorena I, Ganly P, Mudie L, McLaren S, O'Meara S, Raine K, Jones DR, Teague JW, Butler AP, Greaves MF, Ganser A, Döhner K, Schlenk RF, Dohner H. & Campbell PJ (2016) Genomic classification and prognosis in acute myeloid leukemia. New England Journal of Medicine, 374, 2209–2221.

Patel BJ, Przychodzen B, Thota S, Radivoyevitch T, Visconte V, Kuzmanovic T, Clemente M, Hirsch C, Morawski A, Souaid R, Saygin C, Nazha A, Demarest B, LaFramboise T, Sakaguchi H, Kojima S, Carraway HE, Ogawa S, Makishima H, Sekeres MA & Maciejewski JP (2017) Genomic determinants of chronic myelomonocytic leukemia. Leukemia, 31, 2815–2823. [PubMed: 28555081]

Pederiva C, Böhm S, Julner A. & Farnebo M. (2016) Splicing controls the ubiquitin response during DNA double-strand break repair. Cell Death and Differentiation, 23, 1648–1657. [PubMed: 27315300]

Pérez C, Martínez-Calle N, Martín-Subero JI, Segura V, Delabesse E, Fernandez-Mercado M, Garate L, Alvarez S, Rifon J, Varea S, Boultwood J, Wainscoat JS, Cruz Cigudosa J, Calasanz MJ, Cross NCP, Prósper F. & Agirre X. (2012) TET2 mutations are associated with specific 5-methylcytosine and 5-hydroxymethylcytosine profiles in patients with chronic myelomonocytic leukemia. PLoS ONE, 7, e31605. [PubMed: 22328940]

Shen S, Park JW, Lu Z, Lin L, Henry MD, Wu YN, Zhou Q. & Xing Y. (2014) rMATS: robust and flexible detection of differential alternative splicing from replicate RNA-Seq data. Proceedings of the National Academy of Sciences of the United States of America, 111, E5593–E5601. [PubMed: 25480548]

Such E, Cervera J, Costa D, Solé F, Vallespí T, Luño E, Collado R, Calasanz MJ, Hernández-Rivas JM, Cigudosa JC, Nomdedeu B, Mallo M, Carbonell F, Bueno J, Ardanaz MT, Ramos F, Tormo M, Sancho-Tello R, del Cañizo C, Gómez V, Marco V, Xicoy B, Bonanad S, Pedro C, Bernal T. & Sanz GF (2011) Cytogenetic risk stratification in chronic myelomonocytic leukemia. Haematologica, 96, 375–383. [PubMed: 21109693]

Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J. & Vardiman JW (2008) WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th edn. International Agency for Research on Cancer Press, Lyon, France.

Tong KI, Ota K, Komuro A, Ueda T, Ito A, Anne Koch C. & Okada H. (2016) Attenuated DNA damage repair delays therapy-related myeloid neoplasms in a mouse model. Cell Death and Disease, 7, e2401. [PubMed: 27711078]

Valletta S, Dolatshad H, Bartenstein M, Yip BH, Bello E, Gordon S, Yu Y, Shaw J, Roy S, Scifo L, Schuh A, Pellagatti A, Fulga TA, Verma A. & Boultwood J. (2015) ASXL1 mutation correction by CRISPR/Cas9 restores gene function in leukemia cells and increases survival in mouse xenografts. Oncotarget, 6, 44061–44071. [PubMed: 26623729]

Vardiman JW, Ergen Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellström-Lindberg E, Tefferi A. & Bloomfield CD (2009) The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood, 114, 937–951. [PubMed: 19357394]

Wray J, Williamson EA, Singh SB, Wu Y, Cogle CR, Weinstock DM, Zhang Y, Lee SH, Zhou D, Shao L, Hauer-Jensen M, Pathak R, Klimek V, Nickoloff JA & Hromas R. (2013) PARP1 is required for chromosomal translocations. Blood, 121, 4359–4365. [PubMed: 23568489]

Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, Sato Y, Sato-Otsubo A, Kon A, Nagasaki M, Chalkidis G, Suzuki Y, Shiosaka M, Kawahata R, Yamaguchi T, Otsu M, Obara N, Sakata-Yanagimoto M, Ishiyama K, Mori H, Nolte F, Hofmann WK, Miyawaki S, Sugano S, Haferlach C, Koeffler HP, Shih LY, Haferlach T, Chiba S, Nakauchi H, Miyano S. & Ogawa S. (2011) Frequent pathway mutations of splicing machinery in myelodysplasia. Nature, 478, 64–69. [PubMed: 21909114]

Zereshkian A, Leyton JV, Cai Z, Bergstrom D, Weinfeld M. & Reilly RM (2014) The human polynucleotide kinase/phosphatase (hPNKP) inhibitor A12B4C3 radiosensitizes human myeloid leukemia cells to Auger electron-emitting anti-CD123 111In-NLS-7G3 radioimmunoconjugates. Nuclear Medicine and Biology, 41, 377–383. [PubMed: 24637100]

Zhang Y. & Jasin M. (2011) An essential role for CtIP in chromosomal translocation formation through an alternative end-joining pathway. Nature Structural & Molecular Biology, 18, 80–84.

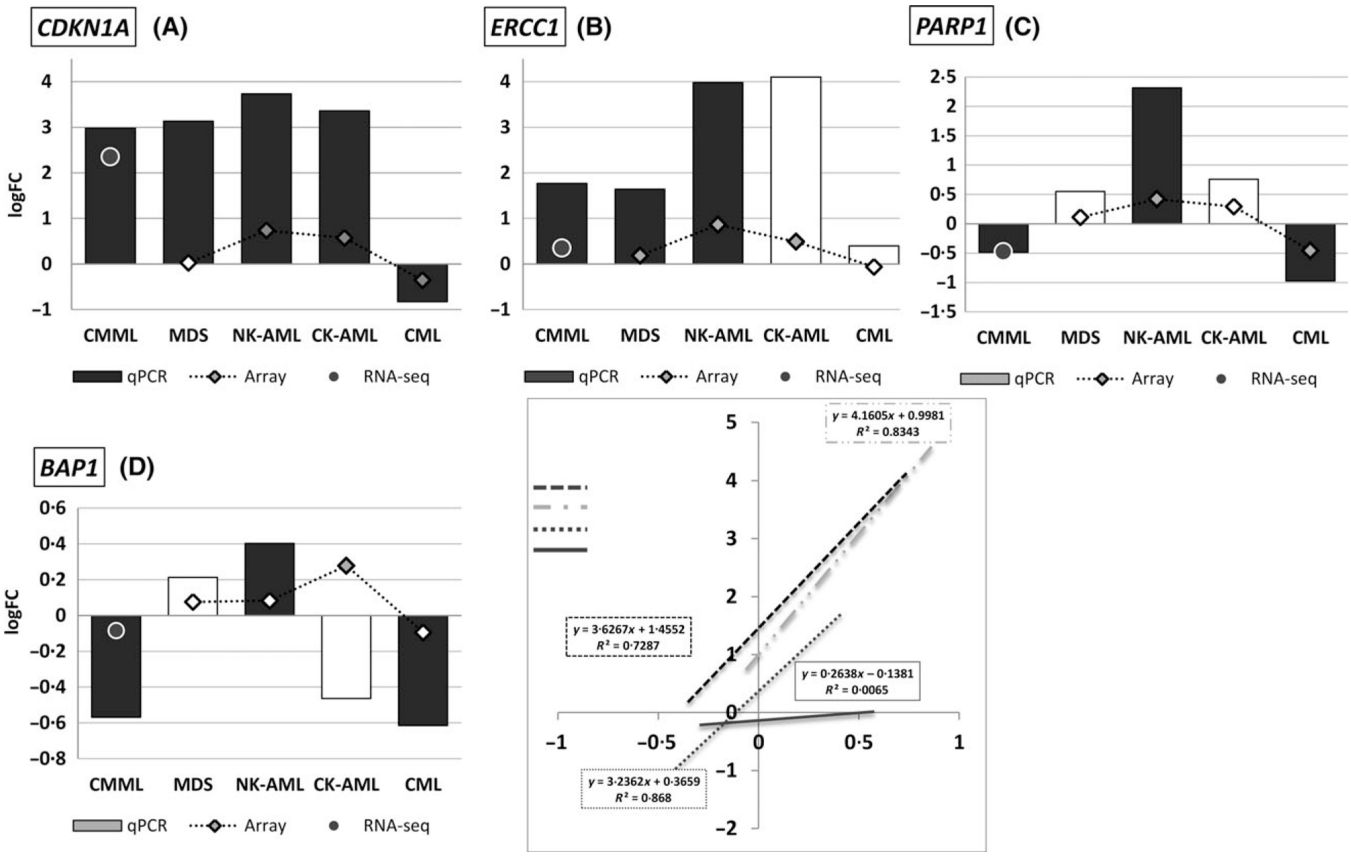

Fig 1.

CDKN1A, ERCC1, PARP1 and *BAP1* misregulation is validated in an independent CMML cohort and their behaviour through myeloid disorders replicated by both direct and global comprehensive techniques. The columns in each gene-plot (A–D panel) depict the RT-qPCR log2FC of, from left to right, a validation cohort of 74 CMML patients, 70 MDS, 53 AML, 41 normal karyotype AML, 12 complex karyotype AML and 25 CML cases. The symbols in each gene-plot represent levels of gene expression extracted from global means: • illustrates the log2FC of our CMML RNA-Seq data discovery cohort; ◊ depicts the log2FC of MILE Study Microarray data including 206 MDS, 351 normal karyotype AML, 48 complex karyotype AML, and 76 CML cases. Grey columns and/or symbols detail statistically significant differences *versus* controls (all healthy bone marrow donors: 9 for RNA-Seq data, 15 for RT-qPCR data, 73 for microarray data). White columns and/or symbols; not statistically significant. (E) Plot showing the relationship between RT-qPCR (Y axis) and Microarray (X) data for each gene in every tested subset of myeloid disorder. Lack of correspondence of *BAP1* data is explained by a marked discrepancy in complex karyotype AML cases. Note: nkAML = non t(15;17) AML cases. CK-AML, complex karyotype acute myeloid leukaemia; CML, chronic myeloid leukaemia; CMML, chronic myelomonocytic leukaemia; logFC, log fold change; MDS, myelodysplastic syndrome; NK-AML, normal karyotype acute myeloid leukaemia (non t(15;17) AML); qPCR, quantitative real time polymerase chain reaction; RNA-Seq, RNA sequencing.

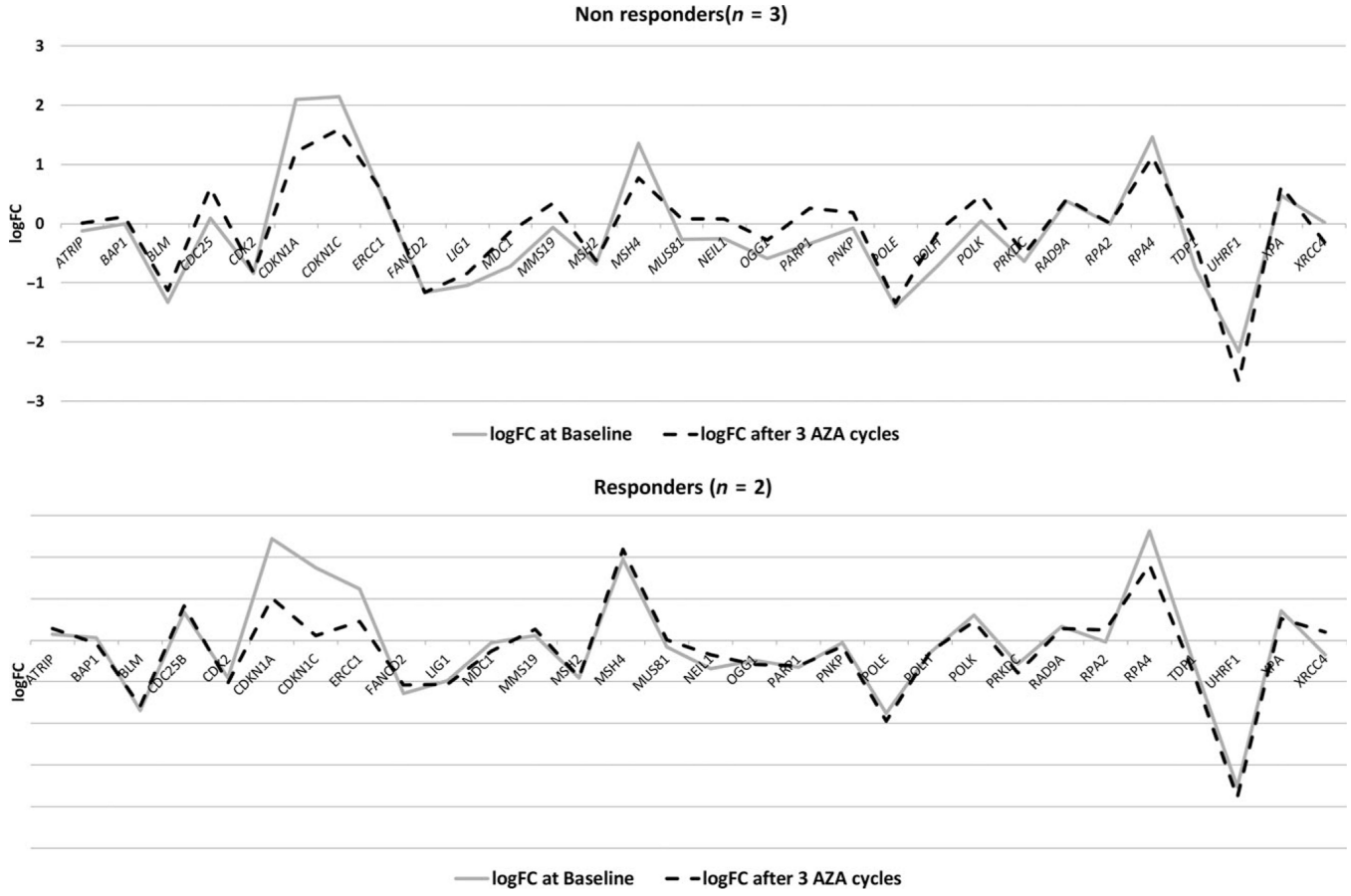

Fig 2.

Expression dynamics of DNA Repair DEGs after 3 cycles of azacitidine. (A) Line graph representing the differential expression *versus* controls (DE) of 3 CMML patients who failed to achieve any response to azacitidine treatment. The grey line depicts DE at baseline; the dashed black line indicates DE after 3 cycles of azacitidine. (B) Line graph representing the DE of 2 CMML patients who achieved complete response to azacitidine treatment. The grey line depicts DE at baseline; the dotted black line indicates DE after 3 cycles of azacitidine. AZA, azacitidine; CMML, chronic myelomonocytic leukaemia; DEGs, differentially expressed genes; logFC, log fold change.

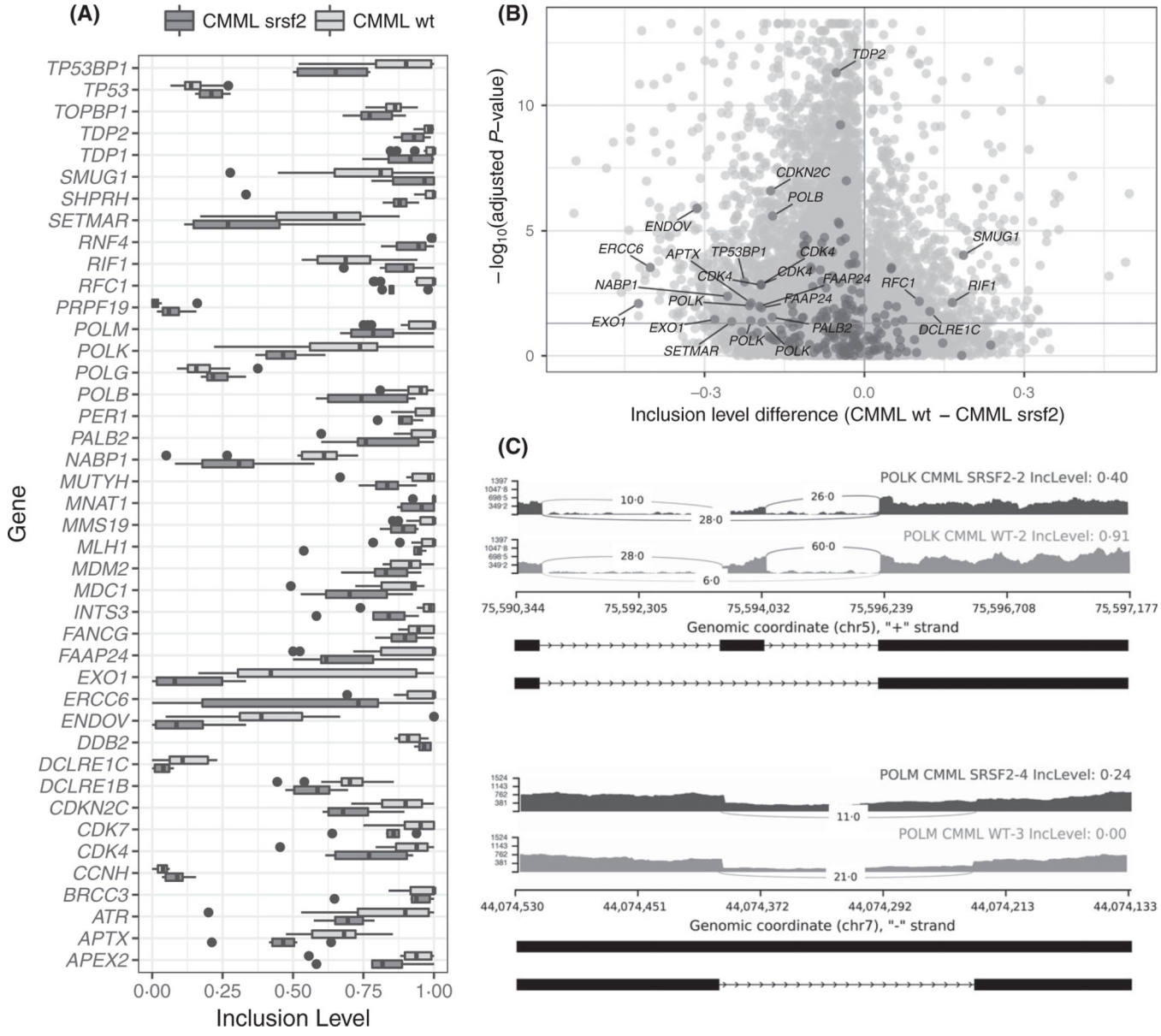

Fig 3.

Aberrant splicing of DNA repair genes in *SRSF2*-mutated CMML. (A) Box plots indicating significant exon skipping events for 42 DNA repair genes, comparing average exon usage for *SRSF2*-mutated (n = 7; grey boxes) *versus* spliceosome mutation wild type (WT, n = 14; white boxes) chronic myelomonocytic leukaemia (CMML) cases from our validation cohort RNA-Seq dataset. Exon skipping events were considered significant if occurring with inclusion difference level ≥0·05 and false discovery rate <0·05. Where multiple skipped exon events occurred in the same gene, only the event displaying greatest magnitude of difference was included. Boxes represent the interquartile range, with median inclusion level indicated by the bold bar; whiskers and outliers are plotted using the Tukey method. (B) Volcano plot indicating all significant exon skipping events comparing *SRSF2*-mutated *versus* spliceosome mutation wild type CMML cases. Events involving DNA repair genes

are highlighted in deep grey; selected significant events are labelled. (C) and (D) Sashimi plots comparing RNA-Seq reads across splice junctions for two illustrative patients, for two exemplar alternative splicing events: (C) exon skipping event in *POLK* and (D) intron retention event in *POLM*. SRSF2-mutated patients are indicated by grey tracks; spliceosome mutation wild type patients are represented by black tracks. Relevant genomic coordinates and intron/exon tracks are provided beneath each plot.

Table I.

Characteristics at baseline of CMML patients included in the RNA-Seq cohort (n = 27) and in the extended validation cohort (n = 74).

Variable	RNA-Seq discovery cohort (n = 27)	Extended validation cohort (n = 74)
Age, years, (median, range)	70 (63–83)	75 (44–91)
Sex, male/female, %	67/33	62/38
WHO subtype, %		
CMML-0	42	48
CMML-1	35	37
CMML-2	23	15
FAB subtype, %		
CMML-MD	54	63
CMML-MP	46	37
Leucocytes, × 10^9/l (median, IQR)	17·5 (24–99)	12·5 (24–89)
Monocytes, × 10^9/l (median, IQR)	2·9 (1–156)	2·1 (1–138)
Haemoglobin, g/l (median, IQR)	94 (77–156)	108 (69–168)
Platelet count, × 10^9/l (median, IQR)	147 (18–844)	150 (17–874)
Extramedullary disease (%)	37	27
Cytogenetic, abnormal karyotype (%)	11	9
Bone marrow blasts (median, IQR)	6 (1–19)	5 (1–19)
Follow-up, months (median, IQR)	24 (2–156)	24 (1–156)
Death (%)	59	50
Leukaemic transformation (%)	15	15

CMML, chronic myelomonocytic leukaemia; FAB, French-American-British; IQR, interquartile range;MD, myelodysplastic; MP, myeloproliferative; RNA-Seq, RNA sequencing; WHO, World Health Organization.