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Low frequency *TP53* hotspot mutation contributes to chemoresistance through clonal expansion in acute myeloid leukemia

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Abstract

TP53 mutations (*TP53mut*) in AML patients associate with poor prognosis that may affect therapy and outcome. In addition to *TP53 mut* patients, TCGA AML patient sequencing data shows that there are around 3% of patients have detectable low frequency *TP53mut* reads. Importantly, these patients showed worse outcome as compared to the *TP53* wild type (*TP53wt*) patients. We have studied the effect of low frequency *TP53mut* in two AML cell lines, OCI-AML2 and MV4–11. Both cells have low frequency single hotspot *TP53mut*. Interestingly, the resistant cells derived from both lines have homogeneous *TP53mut*. *TP53mut* clones isolated from the parental cells also show increased chemoresistance potential and have higher population of leukemia stem cell (LSC) marker positive cells, a characteristic of chemoresistant cells. When mixed with *TP53wt* cells, the *TP53mut* cells show survival advantage suggesting its potential to develop chemoresistance. We previously showed that histone deacetylase inhibitor Romidepsin can re-sensitize chemoresistant cells by eradicating LSC marker positive cells. Here we further show that Romidepsin can reactivate p53 targeted genes which are dysregulated in *TP53mut* cells and preferentially targets *TP53mut* subpopulation. Therefore, our study shows that low frequency *TP53mut* is linked to chemoresistance and shed lights on therapeutic strategies for treatments on chemoresistance.

Keywords

TP53 mutation; chemoresistance; AML

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Conflict of Interest

All authors declare no conflict of interest.

Introduction

Chemoresistance is a major burden for the treatment of many cancers, including acute myeloid leukemia (AML). Overall survival of AML patients, despite intensive treatment with high-dose chemotherapy, stem cell transplantation, and radiation therapy, remains poor with the five-year overall survival less than 30%. The major burden for therapy is chemoresistance. AML is a clonal disease and chemoresistant cells may either evolved from the expansion of a subclone of the primary cells or from the clone gained additional mutations during therapy¹. The mechanism underlying resistance remains to be elucidate.

Tumor suppressor p53 is the first in line guardian of the genome in cells. In response to cellular stress, such as DNA damage, UV irradiation or serum deprivation, p53 is stabilized and promotes cell-cycle arrest, apoptosis, or other anti-proliferative programs^{2, 3}. p53 is a transcription factor and numerous p53-responsive target genes, such as *CDKN1A*, *GADD45*, *MDM2*, and *BAX*, contain the p53 consensus sequence in their respective promoters⁴⁻⁸. *TP53* is the most frequently mutated gene in human cancers. Most *TP53mut* occur in the DNA binding domain and disrupt its transcriptional activity for its wild-type target genes, therefore preventing stress responses and enabling aberrant proliferation and survival of *TP53* mutated (*TP53mut*) cells. The mutant p53 protein is often stabilized due to reduced interaction with negative regulators and degradation^{9, 10}. In addition, these mutant proteins display gain-of-function (GOF) activities beyond *TP53* loss^{11, 12}. Growing evidences show p53 GOF can promote stemness of the cells^{13, 14}, enhancing cell self-renewal¹⁵, promoting cancer stem cell related gene expression in variety of cancers^{13, 16}, which leading to dramatic promotion of invasion, metastasis and chemoresistance through activation of these genes for survival and multidrug resistance^{10, 12, 17}

TP53 mutation occurs in about 30% of therapy related AML/MDS (t-AML/MDS) and less than 10% of de novo AML patients and are strongly associates with the resistance to chemotherapy and shorten survival^{1, 18-21}. In this study, we found that patients with low frequency *TP53mut* also show poor prognoses. We therefore studied the effect of low frequency *TP53mut* in the development of chemoresistance. Two cell lines from AML patients were analyzed and found with low frequency *TP53mut*. We show *TP53mut* subpopulation of cells exhibits increased drug resistance and the population possessing LSC properties expands while cells gaining chemoresistance. Our results suggest that low frequency mutation is an important factor promoting the development of chemoresistance.

Material and method

Cell lines

OCI-AML2, OCI-AML3, KG-1, MV4-11, THP-1 and Molm-13 were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen and American type Culture Collection repositories and cultured under their instructions. Chemo-resistant cell lines were generated as described previously²². All cell lines were verified by short tandem repeat (STR) analysis and tested for mycoplasma contamination by ICBR sequencing core at University of Florida.

Chemicals and antibodies

Chemicals and antibodies used for this study are listed in Supplementary Table 1

Cell viability

Cell viabilities were tested as previously described²². Briefly, 1×10^4 cells/well were seeded into either vehicle or drug containing culture medium for 72h. The viable cells were tested with MTS assay kit (G5430, Promega). The absorbance was measured at 490nm wavelength. Cell viability was calculated by the comparison of the absorbance reading obtained from treated versus control cells after subtraction of the background.

RNA-sequencing analysis

RNA libraries were prepared using the TruSeq RNA sample prep kit (Illumina, San Diego) and sequenced using Illumina HiSeq 2000 Sequencer (Illumina). RNA-seq data were processed using Tophat-Cufflinks pipeline²³. Gene set enrichment analysis was performed with GSEA software²⁴. Variants detection and *TP53* mutation sequencing data visualization was carried out by Partek flow.

Interested genes were confirmed by Realtime RT-PCR. Primers are listed in Supplementary Table 2.

Raw data and normalized gene expression data are deposited in the Gene Expression Omnibus database under accession number GSE108142 and GSE114649 as previously described²².

Chromatin Immunoprecipitation (ChIP)

ChIP assay was performed as described previously^{25, 26}. Briefly, 5×10^6 cells/sample were cross-linked by 1% formaldehyde and quenched with 0.125M glycine. Cells were sonicated to obtain approximately 300bp-500bp chromatin fragments. And subsequently immunoprecipitated with indicated antibodies or IgG as control. The purified DNA from precipitated chromatin was subjected to qPCR amplification. The primers for ChIP are listed in Supplementary Table 2.

Mouse xenograft studies

Xenograft studies were performed following the protocol approved by the Institutional Animal Care and Use Committee of the University of Florida (IACUC #201909309). MV4-11 single cell clone with *TP53mut* were first infected by lentivirus produced from pMSCV-GFP plasmid, then mixed with MV4-11 parental cells. Then, 1×10^6 mixture cells were injected intravenously into tail vein of 8 to 10-week-old NSG mice (The Jackson Laboratory, 005557). Mice were treated by either 1.2 mg/kg Ara-C or vehicle from day 0 to day 9 and euthanized on day 42.

Statistical analysis

Student's t-test was used to analyze data from gene expression, cell counting, cell viability and in vivo xenograft experiments. Values of $P < 0.05$ were considered statistically significant. Pearson correlation coefficient analysis was carried out by GraphPad Prism 6.

Results

p21 gene expression and protein level were decreased in chemo-resistant cells

In order to study chemoresistance mechanism, Cytarabine (Ara-C) resistant cell lines were generated from parental AML cells, OCI-AML2 and MV4-11 (Fig. S1A, B). RNA-seq analysis was performed to examine global changes in gene expressions in chemoresistant cells. Compared to parental cells, one of highly changed genes in resistant cells is *CDKN1A* (Fig. 1a). RT-PCR and western blot further confirmed that both mRNA and protein level of p21 were reduced in resistant cells (Fig. 1b). Then we investigated whether the decrease of p21 directly links to drug sensitivity. First, we examined the Ara-C IC50 and their p21 expression across 6 human AML cell lines. p21 expression is negatively correlated with drug sensitivity (Fig. 1c). Interestingly, Ara-C treatment failed to induce p21 expression in resistant cells to the level as wild type cells (Fig. 1d). Cell cycle analysis shows cell cycle arrests at G0/G1 stage after Ara-C treatment in sensitive cells but not in resistant cells (Fig. S1C). The patient survival data from TCGA also shows that a lower level of p21 is strongly correlated with poor survival (Fig. 1e). Further, the knocked down of p21 gene expression in OCI-AML2 cells increases drug resistance (Fig. 1f, 1g). Consistently, p21 knock down cells fail to trigger cell cycle arrest upon Ara-C treatment (Fig. S1C). Together, resistant cells have lower p21 expression and Ara-C fails to activate p21 in these cells to trigger cell cycle arrest eventually leading to cell death. Therefore decreased p21 expression in AML chemoresistant cells is linked to Ara-C drug resistance.

TP53mut detected in both resistant cell lines was originated from a small subpopulation of parental cells

p53 is one of the major regulator of *CDKN1A* gene^{27, 28}. Both AML cells, OCI-AML2 and MV4-11, are characterized as *TP53* wild type (*TP53wt*) cells²⁹. We therefore tested if p53 protein level is altered in chemoresistant cells. The result shows there is in fact, an increase of protein level of p53 in drug resistant cells, instead of decrease (Fig. 2a). In addition, RNA-seq results show gene expressions of a panel of p53 target genes were decreased in resistant cells (Fig. 2b). Giving the fact that p53 protein is expressed but p53 target gene is not activated, we suspect that *TP53* gene is mutated in the resistant cells. Sanger sequencing results confirmed that both resistant cell lines have *TP53mut*, OCI-AML2 R50 has Y220C mutation and MV4-11 R4 has R248W mutation (Fig. 2c). Both mutations are hotspot mutations for *TP53*^{10, 30}. We then performed gene set enrichment assay on gene expression profiles of both chemo-resistant cell lines compared to their parental cells. The result shows that in both resistant cells, changed genes enriched in genes down-regulated in the NCI-60 with mutated *TP53* gene set (Fig. 2d). Furthermore, patient data from TCGA also shows patients with *TP53mut* have lower p21 levels (Fig. S2A). Therefore, lower expression of p53 target genes is linked to *TP53mut* in resistant cells. There are two possible scenarios for *TP53mut* in resistant cells. One is that mutations are induced by Ara-C treatment, and second is that the mutation is originated from a small population of parental cells which was not detectable by conventional Sanger sequencing. Through analysis of our RNA sequencing data, we found that p53 mutant transcripts exist before chemo-drug treatment at 4% in OCI-AML2 cells and 7% in MV4-11 cells, which expanded to nearly 100% after chemotherapy (Fig. 2e). We then turn into clinical data to investigate whether there are population of

patients who considered *TP53wt* are in fact have low frequency *TP53mut* and whether these patients have poor prognosis compare to wild type *TP53* patients. The dataset obtained from TCGA^{20, 31, 32} has 8.5% of AML patients with *TP53* alteration, and the rest of 91.5% of patients have been classified as *TP53wt* (Figure S2B, Left). However, through the analysis of the sequence data on *TP53wt* patients, we found that there are over 3% (6 out of 174 patients) of patients has low frequency *TP53mut* reads under current sequencing depth (Fig. S2B, right panel). The mutation frequency range from 10 to 23 percent, indicating these patients have subclonal cells with *TP53mut* (Table. S3). We therefore divided all AML patients into three groups, *TP53wt*, *TP53mut* and patients with subclonal *TP53mut*. The Kaplan–Meier curve shows patients with *TP53mut* have significantly lower survival rate compared with *TP53wt* group. Similarly, despite lack of survival data on 2 patients, 3 out of 4 patients with subclonal *TP53mut* also have worse survival outcomes (Fig. S2C).

Cells with TP53mut have survival advantage under chemo-drug selection

Based on results from our chemoresistant AML cells and patient clinic data, we hypothesize that *TP53mut* subclone in AML cells has survival advantage and therefore expand after chemotherapy. To test this, we first selected single cell clones which have wild type *TP53* or mutation from parental MV4–11 cells (Fig. 3a). The *TP53* mutation single clones grow in a similar rate as the parental cells (Fig. S3A), but less sensitive upon drug treatment (Fig. 3a, Fig. S3B). Further, we labeled *TP53* mutant cells with GFP, and then mixed with *TP53wt* cells or MV4–11 parental cells to test whether mutated cells have growth advantage under low dose Ara-C treatment. The results show GFP positive *TP53* mutant clone increases cell population dramatically after drug treatment (Fig. 3b, Fig. S3C). Consistent with the in vitro data, when the mixture of GFP *TP53* mutant cells and MV4–11 parental cells were transplanted into irradiated NSG mice and subsequently treated with vehicle or Ara-C, the hCD45/GFP positive *TP53mut* leukemic cell population was significantly expanded in BM and Spleen of recipients upon Ara-C treatment, while percentage of *TP53* WT cells significantly decreased over six weeks period (Fig. 3c). Thus, the data suggest that *TP53* mutant population undergoes clonal expansion in response to the chemotherapy.

Our previous study shows that resistant cells have higher percentage of stem-cell surface marker positive cells²². Since p53 GOF mutant regulates stem cell-associated genes and transcriptional programs^{5, 33, 34}, we further tested whether stem-cell surface marker expression is correlated with *TP53mut*. The result shows that *TP53mut* clone has higher stem cell marker positive cells than *TP53wt* cells (Fig. 3d). Interestingly, the percentage of stem cell maker positive cells in *TP53mut* clone is similar to the resistant cells which have homogeneous *TP53mut* (Fig. S3D). Therefore, these results suggest that *TP53mut* contribute to stem-like phenotype and chemoresistance.

TP53mut results in a reduction of GADD45A and other p53 target genes, and their decrease contribute to drug resistant

GADD45A (Growth Arrest And DNA Damage Inducible Alpha) is a p53 target gene and many evidence implicated that *GADD45A* functions as a stress sensor that results in cell cycle arrest, DNA repair or apoptosis^{35, 36}. However, its role for chemoresistance is not studied. We first examine the gene expression and protein level in chemoresistant cells.

GADD45A was decreased in both gene expression and protein level in resistant cells, consistent with the notion that p53 activity is required for GADD45A gene activation (Fig. 4a). The NCI-60 human tumor cell Ara-C drug response data³⁷ shows negative correlation of GADD45A expression level and drug resistance (Fig. 4b). In addition, we tested the correlation of drug sensitivity and GADD45A expression level across six AML cell lines and seen consistent results (Fig. S4A). These results implicate that lower GADD45A level is linked to chemoresistance. Importantly, Ara-C failed to activate GADD45A expression in resistant R4 cells compare to the level elevated in sensitive cells (Fig. 4c). Interestingly, *TP53mut* clone isolated from parental cells, which showed higher resistance to Ara-C, also has lower level of GADD45A expression and fails to response to Ara-C to levels compare to parental cells (Fig. 4c). Further, the knocked down of GADD45A in OCI-AML2 cells increases drug resistance (Fig. 4d). These data shows that reduction of GADD45A in *TP53mut* cells contributes to chemoresistance.

We also examined whether Ara-C also failed to activate other p53 target genes in resistant cells and *TP53mut* parental cell clones. polo-like kinase 2 (PLK2) and BAX protein has been shown are direct targets of p53 and are linked to chemoresistance³⁸⁻⁴¹. Our study shows that the expression of both genes are reduced in resistant cells as well as *TP53mut* cells. In addition, the expression of both genes fail to be fully activated by Ara-C in these cells (Fig. S4B). Therefore, mutated p53 failed to activate its target genes which are important for cell death and chemosensitivity.

TP53mut causes reduction of p53 recruitment at both p21 and GADD45A promoters leading to a decrease of histone acetylation in chemoresistant cells

As both *TP53mut* in resistant cell lines are at DNA binding domain, we next want to test if dysregulation of downstream p53 target genes result from the loss of its chromatin recruitment. Chromatin Immunoprecipitation was performed in both chemoresistant cell lines and their parental cell lines. There is a significant reduction of p53 recruitment at p21 promoter regulatory regions (Fig. 5a, Fig. S5) and p53 binding site near *GADD45A* promoter (Fig. 5b). Consistent with low p53 recruitment, histone acetylation at same regions is also reduced, leading to repression of gene expression (Fig. 5a, b and Fig. S5).

Romidepsin can restore genes dysregulated by TP53mut by increase histone acetylation and preferentially targets TP53mut cells

It has been shown HDAC inhibitors (HDACi) can elevate p21 expression^{42, 43}. Therefore we are interested to examine whether HDACi can restore p21 gene expression in *TP53mut* cells. The HDAC1/2 inhibitor Romidepsin treatment activates p21 expression in both parental OCI-AML2 cells and chemoresistant cells (Fig. 6a). Similarly, GADD45A gene expression increased upon Romidepsin treatment (Fig. 6a). We further demonstrate that Romidepsin restores gene expression by increasing histone acetylation at gene promoter regions (Fig. 6b, Fig. S6A). Interestingly, p53 recruitment was not restored by Romidepsin treatment (Fig. S6B, S6C). We then further examined whether Romidepsin can rescue p53 targeted genes in general. Gene set enrichment assay was performed with RNA seq data from resistant cells with or without Romidepsin. Interestingly, Romidepsin activated genes are enriched in genes downregulated by *TP53mut* in NCI-60 cancer cell data set (Fig. 6c).

Heatmap shows majority of representative p53 target genes that are decreased in chemoresistant cells were reactivated by the Romidepsin treatment (Fig. 6c).

Since Romidepsin can resensitize resistant AML cells in response to chemotherapy²², and restore the expression of p53 target genes in *TP53mut* cells, we next wanted to investigate how Romidepsin influences *TP53* mutant subpopulation in AML cells. GFP positive *TP53mut* cells isolated from MV4–11 parental cells were mixed with MV4–11 *TP53* WT cells and then treated with low dose Romidepsin for 4 days. The percent of GFP positive cells were significantly reduced (Fig. 6d), indicating Romidepsin preferentially targets *TP53mut* cells. In contrast, hypomethylating agent decitabine can not target *TP53mut* cells as efficient as Romidepsin under similar cell killing rate (Fig. S7). Therefore the Romidepsin treatment potentially can suppress the clonal expansion of *TP53mut* cells and prevent or slowdown the development of chemoresistance during chemotherapy.

Discussion

In this study, we investigated genes that are important for AML chemoresistance. We found the expression of two p53 target genes, such as *CDKN1A*, *GADD45A*, are decreased in both resistant AML cell lines which are created from two parental sensitive cell lines, OCI-AML2 and MV4–11. p21 and GADD45A expression level directly affects drug sensitivity of AML cells. Further, RNA-seq result reveals that in fact, most of p53 targeted genes are repressed in resistant cells. These results prompted us to investigate the role of p53 in drug resistance in these cells. Since these two cell lines were characterized as *TP53wt*²⁹, we first considered whether p53 protein level is reduced in resistant cells. To our surprise, p53 protein level is not reduced, instead, is increased. Since *TP53mut* often cause increase of p53 protein level due to reduction of interaction with negative regulators^{10, 11}, we look into whether p53 is mutated in resistant cells. The Sanger sequencing results show that although parental cell lines are *TP53wt*, both resistant cells derived from the parental lines have point hotspot mutation at p53 DNA binding domain. We then investigated whether the mutation is originated from a subclone of parental cells. The RNA-seq result shows that both parental cells have subclonal *TP53mut*. Therefore the result indicates that the chemotherapy does not directly induce *TP53* mutations, instead, subclonal *TP53mut* AML cells have better fitness under chemotherapy and preferentially expended after chemotherapy.

TP53mut occurs in less than 10% of de novo AML but patients with *TP53mut* exhibit lower complete remission rate, higher relapse rate and lower survival rate^{1, 44}. We found six patients with low frequency *TP53mut* from TCGA database and the variant allele frequency ranging from 23% to 10%. Despite lack of survival data on 2 patients, 3 out of 4 patients with subclonal *TP53mut* have worse prognosis compared to wild type p53 patients. Similar results also seen in other studies. It is shown that there's no significant difference of relapse rate and survival rate between high frequency mutation and subclonal mutations⁴⁴. Therefore, subclonal *TP53mut* will also be a predictor of therapy response and patient prognosis. Current targeted sequencing depth may not be sensitive enough for low frequency mutation detection. Future improved ultra-deep sequencing may be needed for subclonal *TP53mut* detection.

Since the collection of patient sample pairs at diagnose and relapse with low frequency *TP53* mutation is not available to us, we isolated subclonal cells from patient derived cell lines and studied the chemoresistance of the mutant clones and also we showed that mutation clone has better fitness to sustain chemotherapy and become populated after chemotherapy. Therefore, these isolated p53 mutant clones from parental cells are good study model for studying how p53 mutation results in clonal expansion which lead to relapse and drug resistant disease.

Our previous study shows that histone deacetylase inhibitor Romidepsin can target stem cell marker positive cells and resensitize cells to chemotherapy. In this study, we show that *TP53mut* cells have higher stem cell marker positive cells, therefore Romidepsin may preferentially targets *TP53mut* cells. We further demonstrate that Romidepsin reactivated p53 targeted genes. The activation is not through the rescue of p53 binding, instead, it is through the increase of promoter acetylation on histone H3 and H4. These results provide further insight that reactivating p53 targeted genes may have therapeutic potential for *TP53mut* AML patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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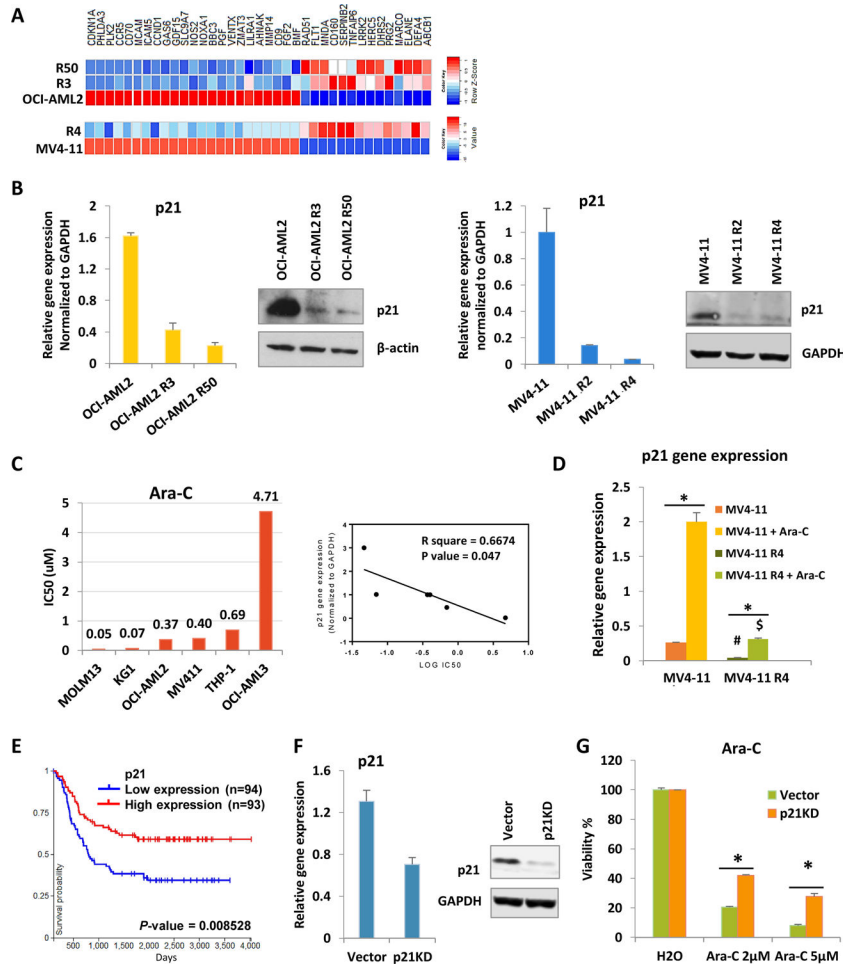


Figure 1. Lower p21 expressions were correlated with drug resistance. (A) Heatmap shows gene expressions of overlapped genes with similar fold change pattern from both resistant cells compared with their parental cells. R3, R50 and R4 represent for cells that can resistant to 3 μM, 50 μM and 4 μM Ara-C. (B) p21 gene expressions and protein levels were tested in OCI-AML2, MV4-11 cells and their resistant lines. (C) Ara-C IC50 were tested across AML cell lines (Left). Pearson correlation coefficient of p21 gene expressions and Ara-C IC50. (D) MV4-11 and resistant cells were treated with either vehicle or 1 μM Ara-C for 24h. #P < 0.05 compared with MV4-11 group. \$P < 0.05 compared with MV4-11 treated with Ara-C group. (E) Kaplan plot for p21 gene expression in AML patients. (F) p21 knockdown efficiency in OCI-AML2 cells. (G) MTS assay after 72h drug treatment with indicated concentrations. Bars indicate the standard error of mean. *P < 0.05

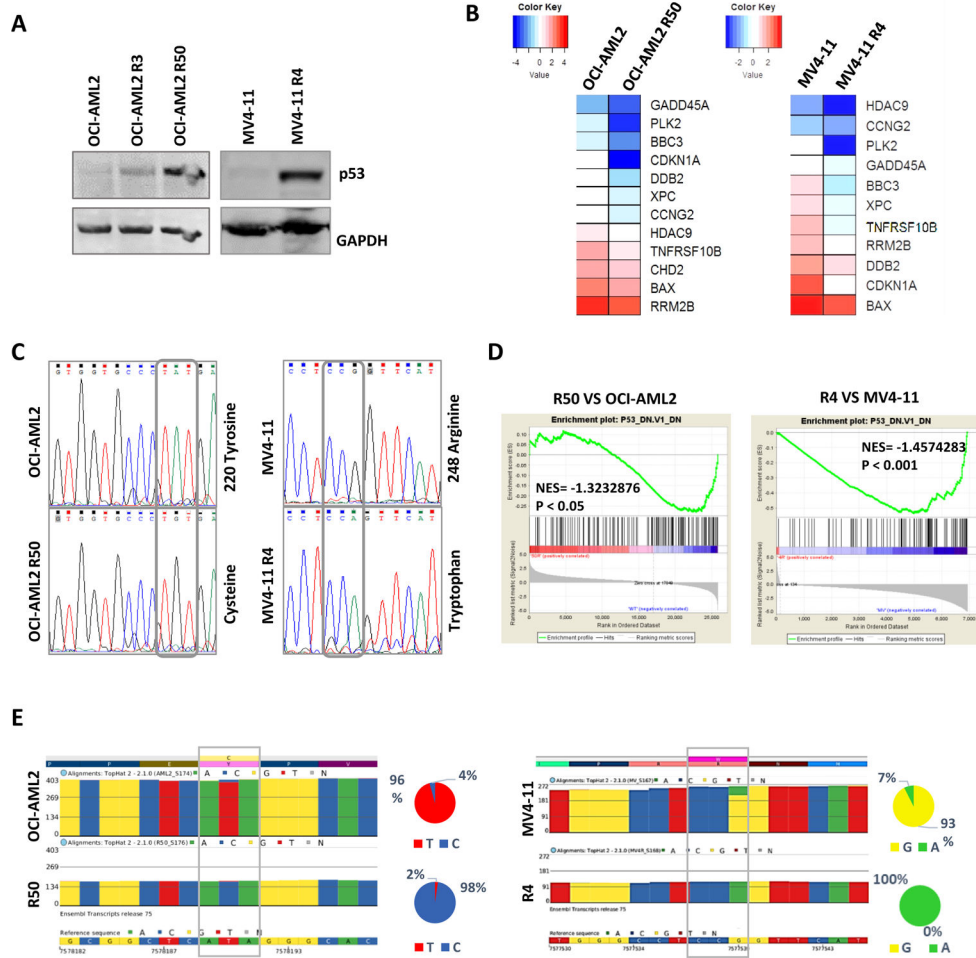


Figure 2. AML cells with *TP53mut* were significantly enriched after chemo drug selection. (A) p53 protein level in AML and chemoresistant cells. (B) p53 target genes in both resistant cells compared to their parental cells. (C) Sanger sequencing were performed on p53 DNA binding domain. Point mutations were found in resistant cells. (D) GSEA shows genes changed in resistant cells are enriched in p53 depletion regulated gene set. (E) Snapshot showing *TP53* sequence reads in indicated cell lines. Top line is protein sequence, bottom is the DNA sequence, bar graph in between is visualized sequencing reads. Percentages of each base at the mutation site in total reads were shown in pie chart.

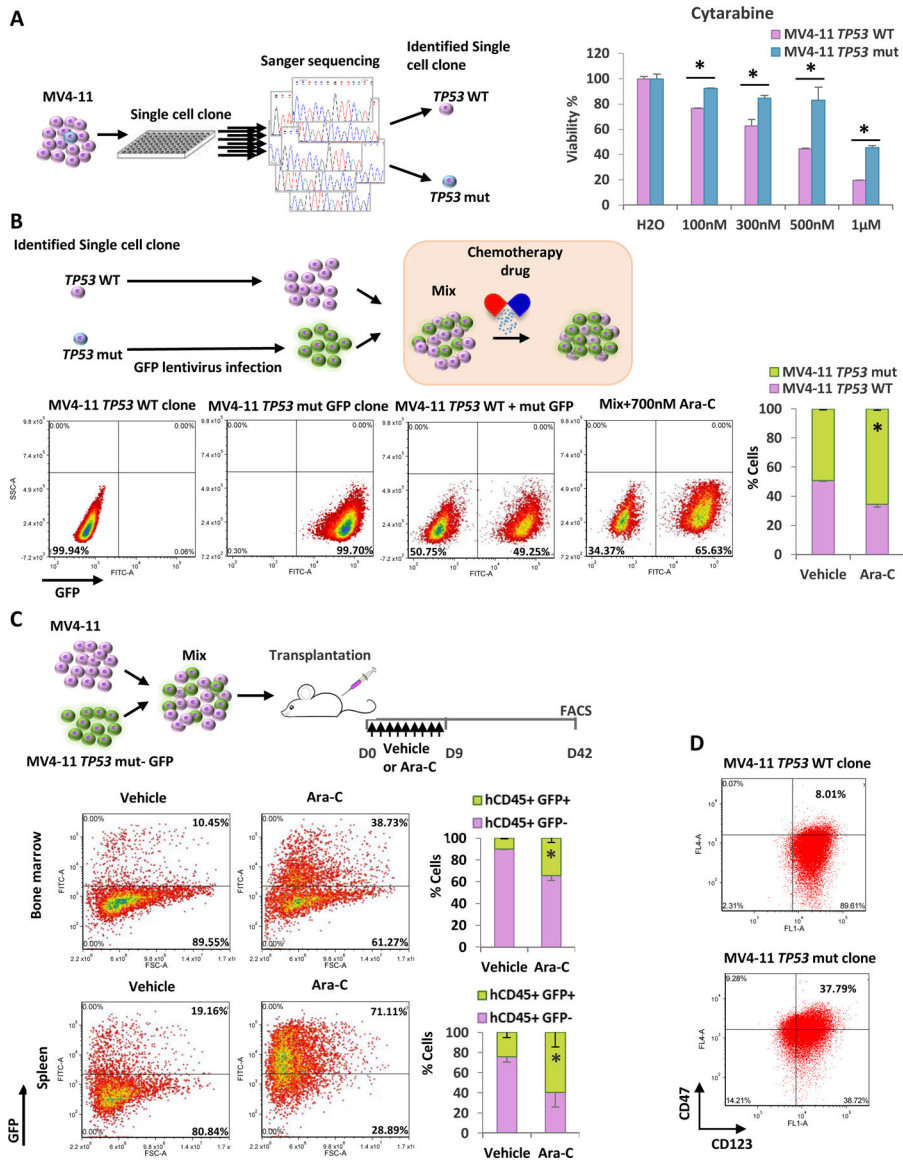


Figure 3. Cells with *TP53mut* have survival advantage under drug pressure and have higher leukemia stem cells percentage.

(A) Left: Flow diagram showing the experimental procedures. Right: MTS assay in *TP53wt* and mutant cells after 72h Ara-C treatment. (B) Top: Flow diagram showing the experimental procedures. Bottom: Single cell clones of MV4-11 *TP53wt* and *TP53mut* labeled with GFP were first tested by FACS separately. Then cells were mixed and treated with either vehicle or Ara-C and tested by FACS on day 5. (C) Top: Flow diagram showing the experimental design. Middle and bottom: cells from bone marrow or spleen were gated for hCD45 positive and tested for GFP signal. Results were summarized in the right panel. Bars indicate the standard error of mean. * $P < 0.05$ (D) Cells were analyzed by FACS for LSC markers.

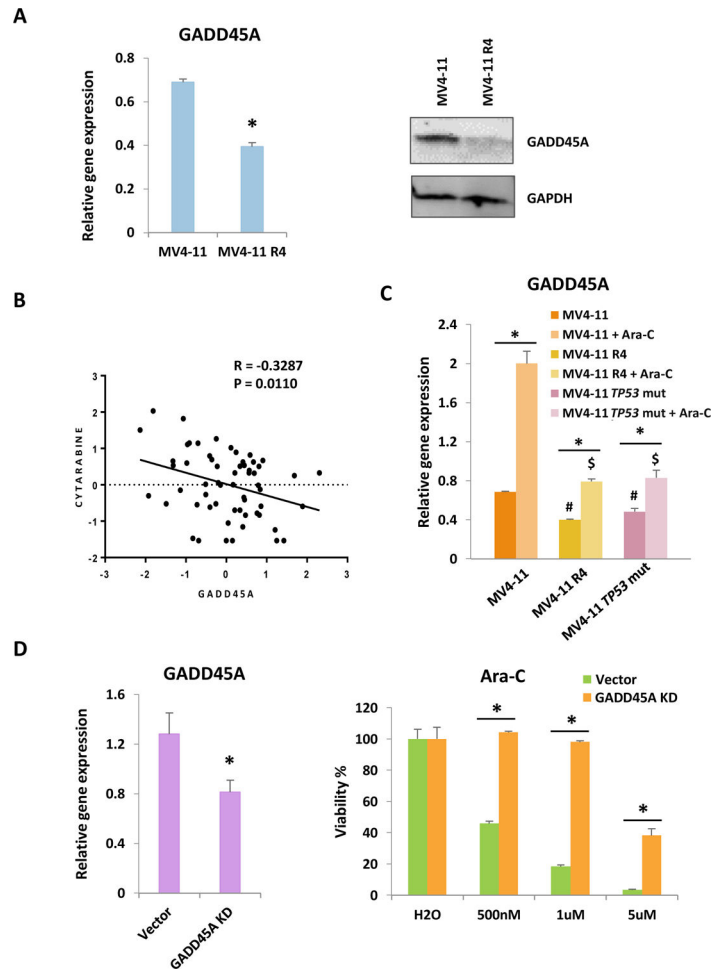
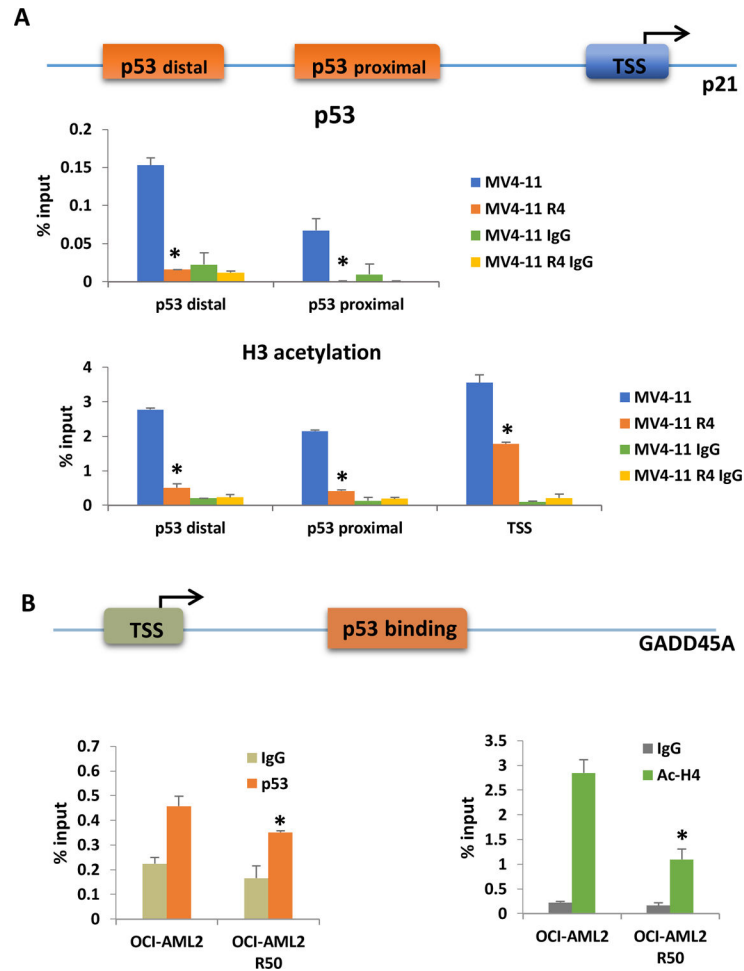


Figure 4.

Decrease of p53 target gene GADD45A also contributes to drug resistance.

(A) Gene expression and protein level were tested in chemoresistant and their parental cells. (B) Correlation of GADD45A gene transcript z-scores and Ara-C compound activities (-log values) z-scores in NCI60 cells. (C) MV4-11, MV4-11 R4 and MV4-11 single cell clone with *TP53mut* were treated with either vehicle or 1 μ M Ara-C for 24h. # $P < 0.05$ compared with MV4-11 group. \$ $P < 0.05$ compared with MV4-11 treated with Ara-C group. (D) shRNA knocking down efficiency of GADD45A in OCI-AML2 cells was tested (left). MTS assay was performed to test drug response in control and knockdown cells after 72h drug treatment (right). Bars indicate the standard error of mean. * $P < 0.05$

**Figure 5.**

p53 mutation causes a reduction of p53 recruitment at both p21 and GADD45A promoters and leading to a decrease of histone acetylation at these regions.

(A) Top: Diagram representation of p21 promoter. p53 distal and p53 proximal are two p53 binding site on p21 promoter, TSS represents for transcription start site. Middle and bottom: ChIP for p53 and ac-H3 in MV4-11 and MV4-11 R4 cells at p21 promoter region. (B) Top: Diagram representation of GADD45A promoter. Bottom: ChIP for p53 and ac-H4 at GADD45A promoter region. Bars indicate the standard error of mean. * $P < 0.05$ compared to parental cell group.

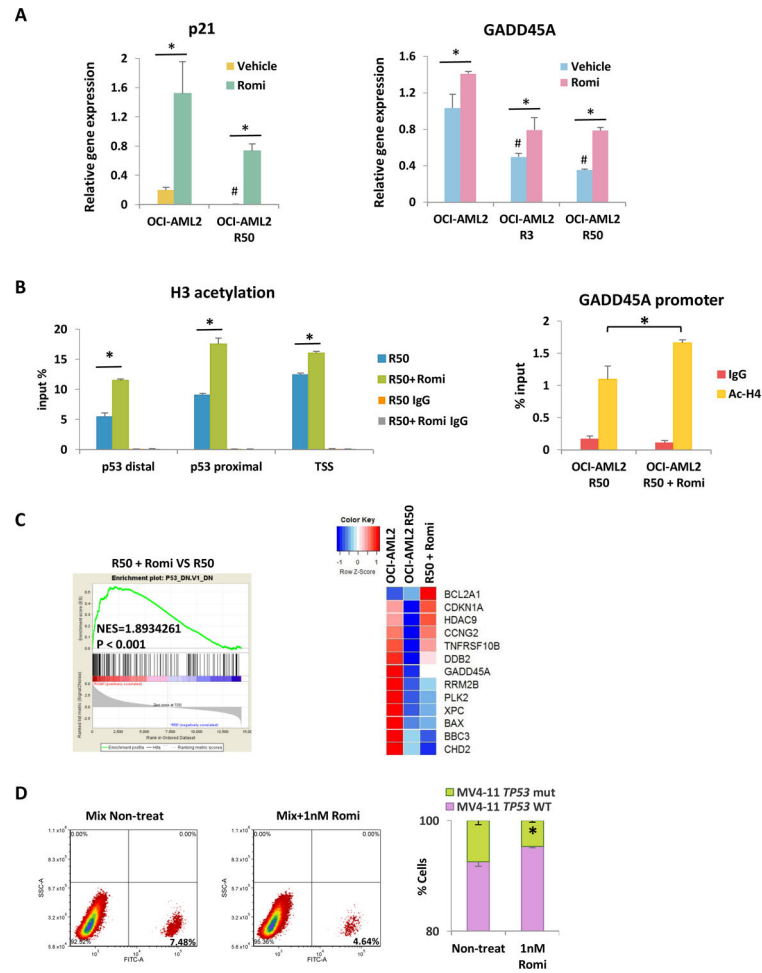


Figure 6. Romidepsin can restore genes dysregulated by p53 mutation by increase histone acetylation and preferentially targets *TP53mut* cells (A) p21 and GADD45A gene expressions upon 24h Romidepsin treatment. # $P < 0.05$ compare to parental cells. (B) ChIP for ac-H3 in OCI-AML2 R50 cells with or without Romidepsin treatment at p21 and GADD45A promoter region. (C) GSEA was performed based on RNA-seq data for Romidepsin treatment in OCI-AML2 R50 cells. Heatmap shows expression of p53 target genes in OCI-AML2, OCI-AML2 50R and OCI-AML2 50R Romidepsin treated cells. (D) Single cell clone of MV4–11 *TP53wt* cells and *TP53mut* labeled with GFP were mixed and treated with either vehicle or 1nM Romidepsin. Cells were tested by FACS analysis on day 4. Bars indicate the standard error of mean. * $P < 0.05$