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Research paper

Low ferroportin expression in AML is correlated with good risk cytogenetics, improved outcomes and increased sensitivity to chemotherapy

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ABSTRACT

Iron metabolism is altered in a variety of cancers; however, little is known about the role of iron metabolism in the biology and response to therapy of acute myeloid leukemia (AML). Here we show that SLC40A1, the gene encoding the iron exporter ferroportin (FPN), is variably expressed among primary AMLs and that low levels are associated with good prognosis and improved outcomes. In particular, core binding factor (CBF) AMLs, which are associated with good outcomes with chemotherapy, consistently have low level of SLC40A1 expression. AML cell lines that expressed relatively low levels of FPN endogenously, or were engineered via gene knockdown, had an increased sensitivity to chemotherapy relative to controls expressing high levels of FPN. Primary FPN^{low} AML bulk cells also had increased sensitivity to Ara-C treatment, iron treatment and the combination of Ara-C and iron relative to FPN^{high} cells. FPN^{low} leukemic stem cells (LSCs) had decreased viability following addition of iron alone and in combination with Ara-C treatment relative to FPN^{high} LSCs. Together these observations suggest a model where FPN mediated iron metabolism may play a role in chemosensitivity and outcome to therapy in AML.

1. Introduction

Iron plays an essential role in many enzymatic and metabolic processes including oxygen transport to tissues as well as cellular respiration [1]. In addition, several specific processes in cellular proliferation are iron dependent including progression through the G1 and S phases of the cell cycle at least in part due to activity of the iron containing enzyme ribonucleotide reductase [2–4,5]. Although essential to tissue and cellular biology for these reasons and others, excess iron is toxic through the generation of reactive oxygen species (ROS) and other mechanisms [6]. Consequently, intracellular iron levels are tightly regulated through complex pathways utilizing specialized proteins including transferrin, ferritin, ferroportin (FPN) and others responsible for iron import and export as well as intracellular distribution of iron [7,8]. Cellular iron uptake relies mainly on the iron-chelating protein transferrin and its carrier protein, the transferrin receptor. The density of the transferrin receptor is higher in cells that require more iron such as cells that are actively proliferating [9]. Ferritin is an important mediator of iron transfer and storage and ferritin concentrations are increased in excess iron states as well as in inflammatory conditions, such as infections or cancer [10]. Ferroportin, encoded by the gene SLC40A1, is also essential for iron homeostasis as it is the only protein responsible for cellular iron export [11,12]. The presence of FPN at the plasma membrane is enhanced by iron loading and down-regulated by the peptide hormone hepcidin 12,13].

The expression and activity of these genes in iron homeostasis is altered in a variety of cancers [14–17]. In breast cancer, gene expression profiles from > 800 women demonstrated that decreased ferroportin gene expression was associated with worsened survival [18]. In addition, iron metabolism genes and iron itself were shown to play important roles in breast cancer biology and may serve as potential therapeutic targets [18,19]. In prostate cancer, FPN as detected by immunohistochemistry was decreased in poorly differentiated prostate cancer relative to more differentiated cancer and benign prostatic hypertrophy, again suggesting an important role for FPN in cancer pathogenesis and biology [20]. In both solid tumors and AML, agents which chelate or otherwise impact iron metabolism can induce differentiation and inhibit cellular proliferation [21–23].

However, beyond these findings, little is known about the biology of iron metabolism or the role that key iron-related proteins play in acute

Abbreviations: AML, acute myeloid leukemia; LSCs, leukemic stem cells; FPN, ferroportin; FCM, flow cytometry; CT, chemotherapy

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Table 1
shRNA targeting sequences.

shRNAs:	Sequences:
Scramble	CCTAAGGTTAAGTCGCCCTCG
SLC40A1 A	CCTGTGTGGAATCATCCTGAT
SLC40A1 B	CGCTGGTGGTACAGAATGTTT
SLC40A1 C	GCATCAGCTATAACTGGAATA
SLC40A1 E	GCCACATTATGTATTTCCGAT

myeloid leukemia (AML), especially in the context of chemotherapy. In this study, we sought to further define the role of the iron exporter FPN in AML including its potential use as a prognostic marker and its impact on AML sensitivity to chemotherapy.

2. Methods

2.1. Analysis of public databases

Analysis of SLC40A1 and other iron metabolism genes for mRNA expression and survival outcomes was performed with data available from The Cancer Genome Atlas (TCGA) [24]. A total of 176 AML patients with complete clinical and gene expression data were included in this analysis. Additional RNA sequencing analysis of SLC40A1 was performed in DeNovo and Relapsed AML samples from 246 patients, accessible through the Beat AML data viewer (Vizome) [25].

2.2. Cell lines and primary specimens

AML specimens were obtained from apheresis products and bone marrow aspirates of patients who gave IRB approved informed consent for sample procurement at the University of Rochester and the University of Colorado in Denver. Cell lines were commercially supplied by DSMZ (MOLM16) or were obtained from the cell line repository of the University Tissue Culture core in Denver (MOLM13).

2.3. shRNA and lentiviral production

shRNA and lentiviral production were performed as previously described [26]. shRNA sequences were cloned into pLKO.1-GFP vector, containing a human U6 promoter to drive shRNA expression and a human PGK promoter to drive GFP expression to label shRNA-expressing cells. The detailed methods for generating lentiviral particles, and infecting AML cells are described here http://www.broadinstitute.org/rnai/public/resources/protocols [27]. The targeting sequences of the shRNAs are shown in Table 1:

2.4. Western blot analysis

Lysates were prepared from flow cytometry sorted GFP^+ enriched MOLM16 populations and probed with an unconjugated mouse anti-SLC40A1 monoclonal antibody (1:1000 dilution) (Novus Biologicals). The levels of proteins for each clone were normalized to GAPDH. Western blots were performed as previously described [28]. The western blot results were imaged on the ChemiDoc Imaging System and visualized in the Image Lab software (BIO-RAD).

2.5. In vitro drug treatments

Primary AML cells or MOLM13 and MOLM16 cell lines were incubated 24–48 hours at 37 °C, 5% CO_2 with 0.5 μM Cytarabine (AraC) (together with Idarubicin (IDA), in some experiments) and/or 25μM-100μM ammonium iron(III) citrate (Sigma Aldrich) or an equivalent volume of vehicle control. While these iron concentrations are different from those encountered in-vivo, the in-vitro iron concentrations we utilized were aimed at generating intracellular iron levels in AML cells in-vitro similar to what would be encountered in-vivo [29]. These concentrations were based on prior studies of the role of iron in cancer in our laboratory and others [30] and differ from in-vivo concentrations due to the varying concentrations of ferritin, serum and other key mediators of iron metabolism. Typically, cells were resuspended at 1×10^6 cells/ml in low serum medium (2% FBS in RPMI) and seeded into 12-well plates in triplicate for each treatment condition.

2.6. Flow cytometry analysis

AML samples were stained as previously described [31,32]. Briefly, cells were resuspended at 10^6 cells/mL in phosphate buffered saline solution (PBS) supplemented with 2% fetal bovine serum (FBS). Cells were stained with fluorescence-conjugated antibodies on ice. After 30 min, the cells were washed, centrifuged and resuspended in cold PBS + 2%FBS before fluorescence-activated cell sorting (FACS) analysis. All antibodies and staining reagents were from BD Biosciences (San Jose, CA) except Ferroportin/SLC40A1 (Novus Biologicals) and CellROX™ for oxidative stress detection (ThermoFisher Scientifics). AML cells were routinely gated through the blast window and expression of $CD45^{dim}$. To assess the effect of chemotherapy and Fe^{2+} on LSC and AML bulk populations, the $CD34^{br}CD38^{lo}$ phenotype was used to define LSCs from most samples. For AML samples that did not express CD34 on their blasts, CD32 together with other AML directed markers that were identified by RNA-Seq, including HLA-DR, CD38 and CD52 were used to define LSC subsets [33].

For apoptosis and ROS detection, cells were incubated with surface antibodies and 5μM CellROX for 30 min. Following this, 5 μl AnnexinV (BD Biosciences) was added to 100 μL of the staining solution for an additional 15 min. Cells were resuspended in AnnexinV buffer (50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4), which contained 1 μg/ml of the cell viability dye 4′,6-diamidino-2-phenylindole (DAPI).

All cell staining and analysis procedures were performed either manually or using high content semi-automated flow cytometry as previously described [34,35]. Flow cytometric analysis was performed on a FACSAria or an LSRII (Becton Dickinson (BD); San Jose, CA) and data were analyzed using FlowJo Software (FlowJo, LLC; Ashland, OR). Cell sorting was performed on FACSAria device equipped with 405 nm violet, 488 nm argon and 633 HeNe lasers (BD).

2.7. Statistics

All statistical analyses were performed using GraphPad Prism 7.0. Unless otherwise specified, unpaired two-tail Student t-tests were performed and a $p < 0.05$ was considered to be significant. Aggregate data was presented as means and standard deviations.

3. Results

3.1. Low level expression of ferroportin in AML is correlated with good risk cytogenetics and better outcomes

To initiate the characterization of FPN in AML, we analyzed the expression of the FPN coding gene SLC40A1 along with other major iron metabolism genes in The Cancer Genome Atlas (TCGA) AML dataset [24] (Fig. 1A and Supplemental Fig. 1A). A wide range of SLC40A1 mRNA expression was observed among this cohort of primary AMLs (Fig. 1A). The expression of SLC40A1 was significantly higher in both poor (n = 42) and intermediate cytogenetic AML risk groups (n = 101) compared with the good cytogenetic risk group (n = 33). Within the good cytogenetic risk group, the Core-Binding Factor (CBF) AMLs (defined by t(8;21) and inv(16), n = 18) [36], were associated with the lowest levels of SLC40A1 expression (Fig. 1A). The other AML subgroup in the good cytogenetic risk group, acute promyelocytic leukemia (APL, characterized by the t(15:17) translocation) had SLC40A1

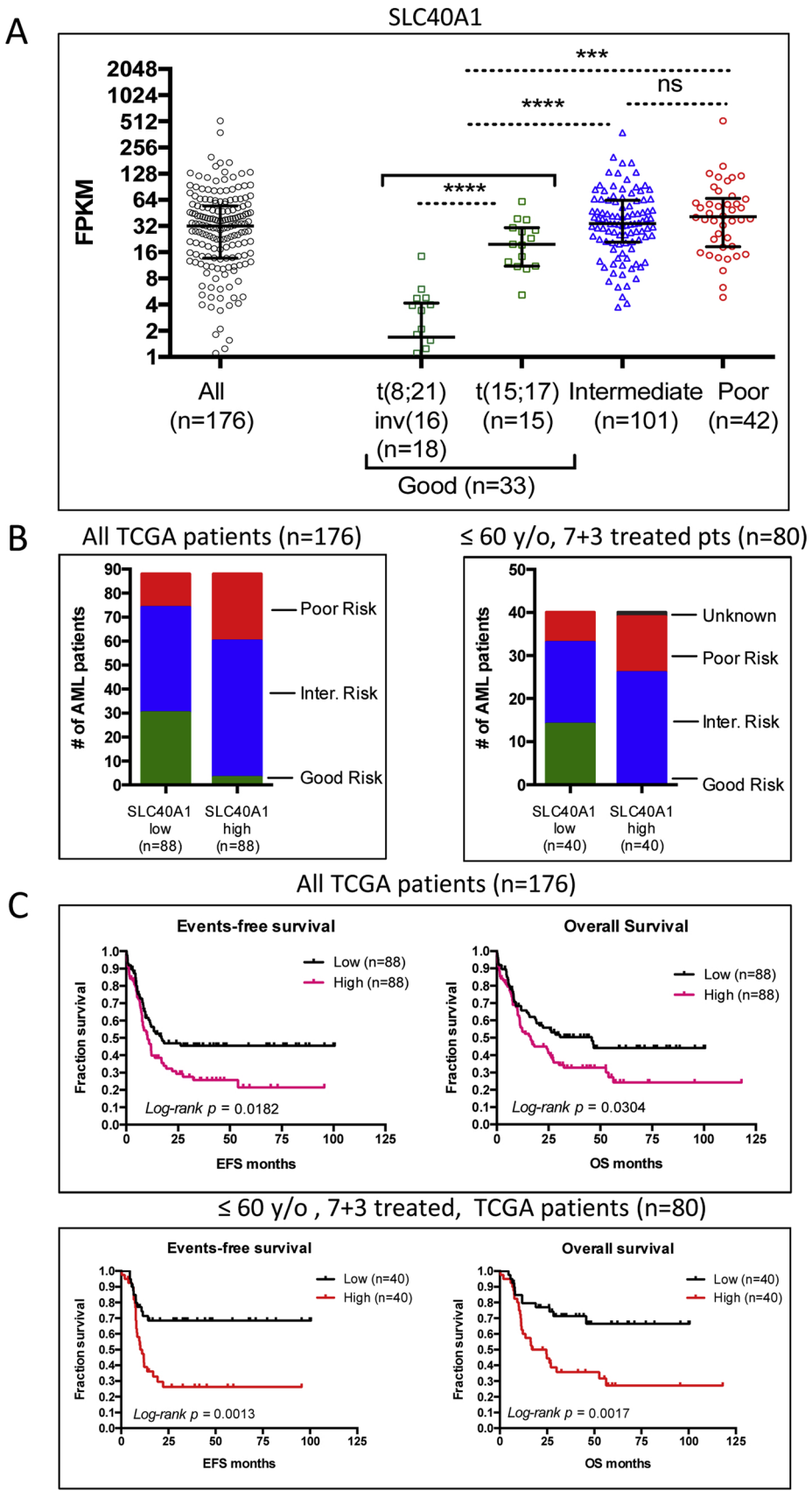

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Fig. 1. Reduced expression of ferroportin (SLC40A1) is correlated with good risk cytogenetics and improved outcomes. (A) Expression of SLC40A1 in all 176 AML specimens and each cytogenetic risk group from the TCGA cohort including good, intermediate and poor risk (3 patients have unknown cytogenetic risk information) [42]. Lines represent median and upper and lower quartiles. A 2-tailed type 3 *t*-test was used to compare the expression of SLC40A1 in each risk group. *** indicates a $p < 0.001$, **** indicates a $p < 0.0001$, ns indicates not significant. The good prognosis group was further subclassified into t(8;21) plus inv(16), commonly referred as Core-Binding Factor AMLs (CBF-AML), and t(5;17) subtypes (****$p < 0.0001$). (B) Distribution of SLC40A1-low and SLC40A1–high AML patients in each cytogenetic risk group (good, intermediate, poor and unknown) from the TCGA cohort. Analysis was conducted within all 176 patients (left panel) and in the subset of 80 patients that were ≤ 60 years old (right panel). (C) Event-free and overall survival of SLC40A1-low and SLC40A1-high AML patients from the TCGA cohort. Analysis was conducted within all 176 patients (top panel) and in the subset of 80 patients that were ≤ 60 years old (bottom panel). In all graphs, the analyses were performed on data from the TCGA Research Network: http://cancergenome.nih.gov/. (D) Flow cytometry dual plots and histogram overlays showing the levels of cell-surface FPN in 2 representative AMLs. Normal marrow elements are displayed as green events and leukemic cells are orange within each sample. (E) Flow cytometry analysis of marrow derived AML samples comparing levels of FPN between Leukemic blasts (orange) and their normal counterparts (Non-leukemic, green) in individual samples (n = 6).

levels similar to the intermediate and poor cytogenetic risk groups. In addition, when the entire cohort of AML specimens was divided into SLC40A1-high (above median) and SLC40A1-low (below median) groups, the SLC40A1-high AMLs enriched for poor and intermediate risk cytogenetics while the SLC40A1-low AMLs contained more good risk cytogenetics. This was also observed in the subset of patients who were younger than ≤60 years old (Fig. 1B) that based on age were candidates for intensive chemotherapy with Ara-C and an anthracycline (i.e. the "7 + 3 regimen") [37]. SLC40A1-low AML patients also had a significantly improved event-free survival (EFS) and overall survival (OS) relative to the SLC40A1-high AML patients in both the total patient group (n = 176 patients) and in the cohort who was ≤60 years old (n = 80 patients) (Fig. 1C). Similar analyses were performed for the heavy and light chains of ferritin (FTH1 and FTL), transferrin (TF), its receptors (TFRC and TFR2) [38] and other genes involved in iron metabolism including divalent metal transporter 1 (DMT1), Zip14, PCBP1 and PCBP2 (Supplemental Fig. 1A–I). Only FTH1 [39] and PCBP2 had any possible prognostic significance and these were relatively modest.

To validate these observations, we analyzed FPN expression in an independent cohort of primary AMLs available at our institution using flow cytometric (FCM) and RNAseq (data not shown) analyses. Wide heterogeneity of FPN expression among different AML specimens was noted, similar to the variation in expression of SLC40A1 in the TCGA dataset (Fig. 1D and E). In addition, in the majority of samples, leukemic blasts demonstrated higher levels of FPN than the non-leukemic populations, suggesting FPN expression may increase upon leukemogenesis in many cases (Fig. 1E).

3.2. Low level expression of FPN leads to increased cytotoxicity in AML treated with chemotherapy

Next we explored the mechanism by which low FPN levels may correlate with relatively better treatment outcome. One possibility is that this is due to an increased sensitivity of AML cells with low FPN levels to conventional chemotherapy. To evaluate this hypothesis, we first tested the impact of FPN levels on sensitivity to chemotherapy treatment in AML cell lines. Wide variation in ferroportin levels in AML cell lines has been previously reported in the publicly available GEO 59808 dataset [40]. From this dataset, the MOLM13 line was identified as a model of low FPN expression and MOLM16 line as a model of high FPN expression. To confirm that these lines would be appropriate models for AML expressing different levels of FPN, we conducted flow cytometric analysis of FPN levels and as predicted, found a lower level of FPN expression in MOLM 13 relative to MOLM 16 both on the cell surface and intracellularly (Fig. 2A–B). Consistent with FPN's function in iron efflux, the intracellular iron in MOLM13 cells was higher than in MOLM16 cells (measured as a decrease in Phen-green SK fluorescence, Fig. 2C) when both were cultured in media with a range of iron concentrations. Fe^{2+} exposure triggered increased levels of surface FPN in MOLM16 cells while no increase was observed in MOLM13 cells (Fig. 2D). Additionally, MOLM16 cells had less cell death following exposure to Fe^{2+} relative to MOLM13 cells (Fig. 2E and F, left half) confirming a biologically protective effect of FPN from iron.

To determine if the differences in FPN levels would result in differential sensitivity to chemotherapy and whether this was affected by iron concentrations, both cell lines were treated with Ara-C alone and Ara-C plus various concentrations of Fe^{2+} (Fig. 2E, F). The low FPN expressing MOLM13 line was more sensitive to chemotherapy alone and in combination with Fe^{2+} compared to the high FPN expressing MOLM16 line.

To test if the decreased chemotherapy sensitivity in MOLM16 was specifically caused by a higher level of FPN, ferroportin expression in MOLM16 cells was knocked down using a lentiviral GFP expressing vector encoding a shRNA against SLC40A1. GFP^+ shRNA-expressing cells from four independent shRNA clones and one scramble shRNA control were isolated by cell sorting and subjected to RNA and protein analyses to evaluate knockdown (KD) levels of SLC40A1 (Fig. 3A, B). Among the four clones tested, clone *C* had $\underline{>}$60% loss of FPN mRNA and protein levels and its FPN expression remained low upon Fe^{2+} exposure (Fig. 3C), confirming that this clone was optimal for subsequent analysis. MOLM16 clone *C* cells had significantly more apoptosis when exposed to Fe^{2+}, Ara-C alone and Ara-C plus Fe^{2+} compared to scramble and ineffective shRNA controls (Fig. 3D). In addition, clone *C* had higher intracellular ROS levels when exposed to the combination of Ara-C, Idarubicin (IDA) and Fe^{2+} compared to controls (Fig. 3E), suggesting ROS increase may play a role in sensitizing low-FPN leukemic cells to chemotherapy in this model.

To determine whether the findings in AML cell lines translated into primary AMLs, 3 samples each of high-FPN and low-FPN expressing AMLs (termed FPN^{high} and FPN^{low} AMLs respectively), as determined by RNA-Seq, were treated with Ara-C alone, different concentrations of Fe^{2+} alone or both in combination. When treated with the chemotherapy agent Ara-C alone, FPN^{low} bulk AML cells lost more viability than FPN^{high} AMLs (Fig. 4A). The addition of iron alone also led to a dose-dependent viability loss in FPN^{low} bulk AML cells but an actual increase in viability in FPN^{high} cells (Fig. 4B). When Ara-C was combined with iron, again a significant decrease in viability was observed in the FPN^{low} bulk AML cells compared to the FPN^{high} cells (Fig. 4C). In addition to the studies performed with bulk leukemia, we also tested whether AML LSCs with different FPN levels were also relatively sensitive to chemotherapy and/or exogenous iron. In these experiments, LSCs were phenotypically defined as shown in Fig. 4D and as described in the Methods section. In these studies, FPN^{low} and FPN^{high} primary AML cells were treated with chemotherapy with or without iron as above and then were analyzed for cell viability by flow cytometric analysis of the phenotypically defined LSC enriched subpopulations, as described in the Methods section (Fig. 4D). No differences between FPN^{low} and FPN^{high} LSC viability was observed with Ara-C treatment

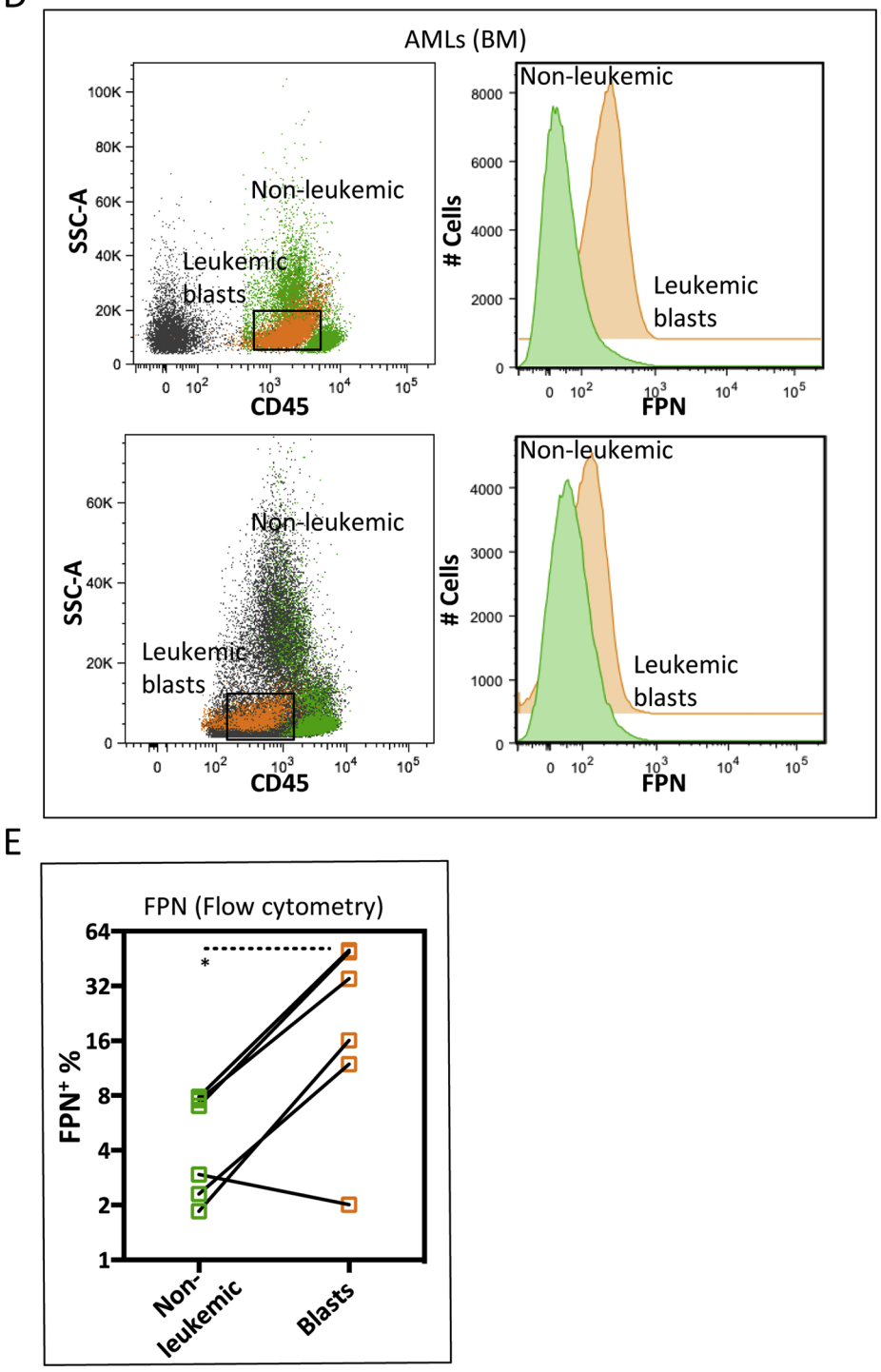


Fig. 1. (*continued*)

alone (Fig. 4E), but in the FPN^{low} LSCs, decreased viability was observed following the addition of Fe^{2+} alone or in combination with Ara-C (Fig. 4F and 4 G). Together these findings suggest that FPN status as well as exogenous iron may influence AML sensitivity to chemotherapy in bulk AMLs and possibly at the LSC level as well.

4. Discussion

In this study, we explored the role of ferroportin (FPN) in AML prognosis and sensitivity to chemotherapy. Analysis of 176 DeNovo AML samples in The Cancer Genome Atlas Study (TCGA) demonstrated that low levels of FPN were associated with good risk cytogenetics as well as with improved overall survival. These associations occurred in younger patients (≤60 years old) as well as the total population. A close association between CBF AMLs in particular and low FPN expression was noted. This is currently unexplained and warrants further studies. One possibility is that CBF itself directly or indirectly reduces FPN expression levels. Alternatively, CBF AMLs may have higher levels

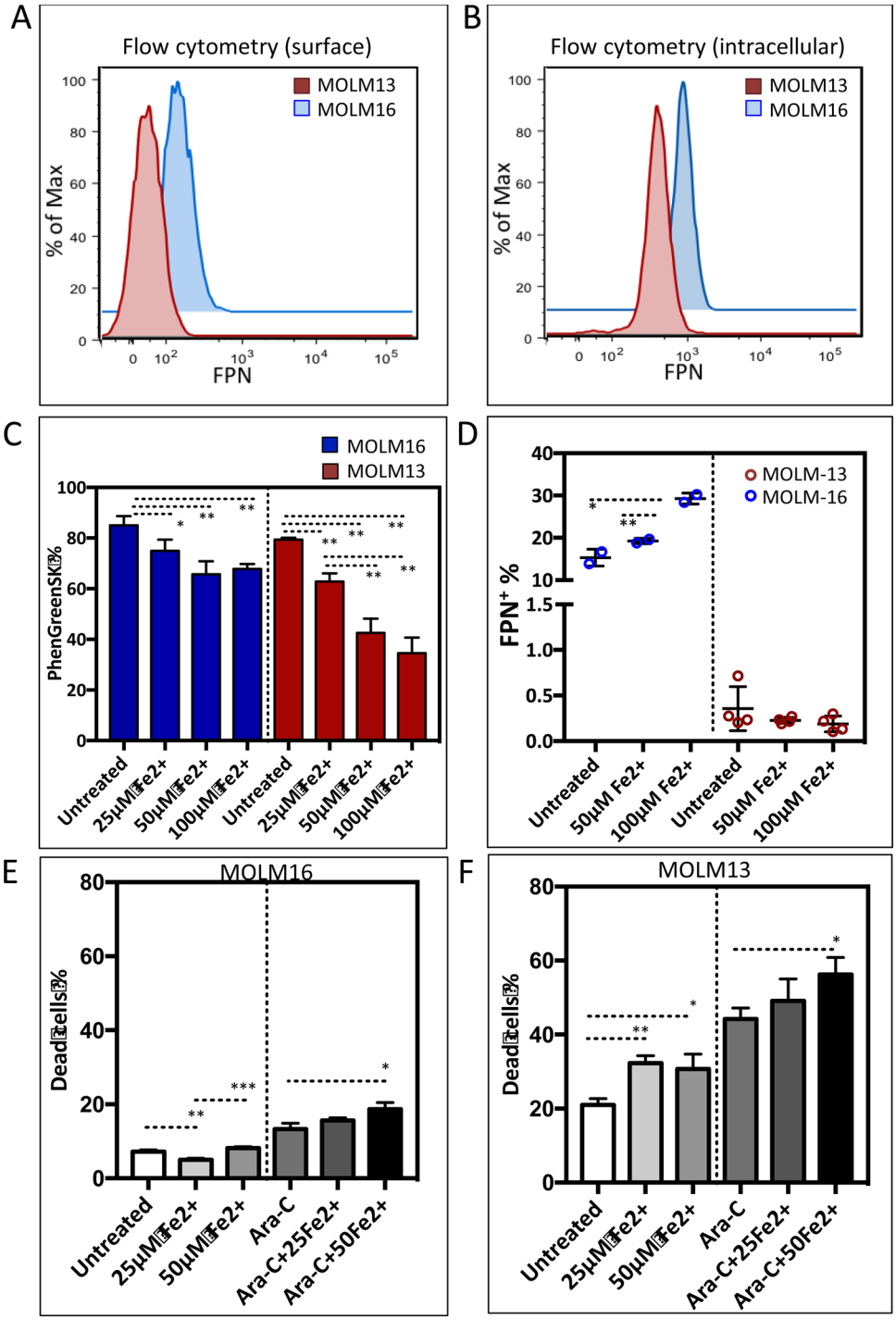


Fig. 2. Characterization of FPN^{high} MOLM16 and FPN^{low} MOLM13 AML cell lines. (A,B) Histogram overlays of intracellular and plasma membrane detection of Ferroportin (FPN) in FPN^{high} MOLM16 cells (blue) and the FPN^{low} MOLM13 cells (red) by flow cytometry. (C) Analysis of intracellular iron levels in MOLM16 and MOLM13 by Phen-green SK fluorescence flow cytometry. Note that *lower* levels of Phen-green fluorescence correspond to *higher* levels of intracellular iron. (D) Cell-surface expression of FPN by flow cytometry in MOLM16 or MOLM13 cells at baseline and after 2 h of 50μM and 100μM Fe^{2+} treatment (n = 2, n = 4; * < 0.05; ** < 0.005). (E, F) Cell viability in MOLM16 (E) and MOLM13 (F) cells treated with 25–50 μM Fe^{2+} plus or minus 0.50μM Ara-C. Viability was measured by flow cytometric analysis of Annexin V/DAPI at 24 h following treatment (n = 3; * < 0.05; ** < 0.005).

of autocrine or paracrine inflammatory cytokines which in turn lead to reduced levels of FPN [21]. In addition, larger prospective studies to confirm the prognostic significance of FPN expression in AML are also warranted. If the association between FPN levels and outcome are confirmed, FPN expression could be used to further enhance the stratification of AML patients to chemotherapy, stem cell transplantation, novel therapies and clinical trials similar to the current use of FLT3, NPM1, CEBPA and other prognostic markers [41,42]. Preliminary data suggests possible trends towards different outcomes when FPN is combined with FLT3, NPM-1 and CEBPA mutational status (see Fig. S2 in the Supplemental figures) but larger cohorts and prospective studies will be needed to determine if this is statistically significant and meaningful.

In contrast to the data reported previously on breast cancer where higher levels of FPN expression were associated with good prognosis, we found an inverse correlation of FPN expression and survival in AML. Possible explanations include differential levels and activity of the FPN regulator hepcidin, other iron regulators or unrelated biologic

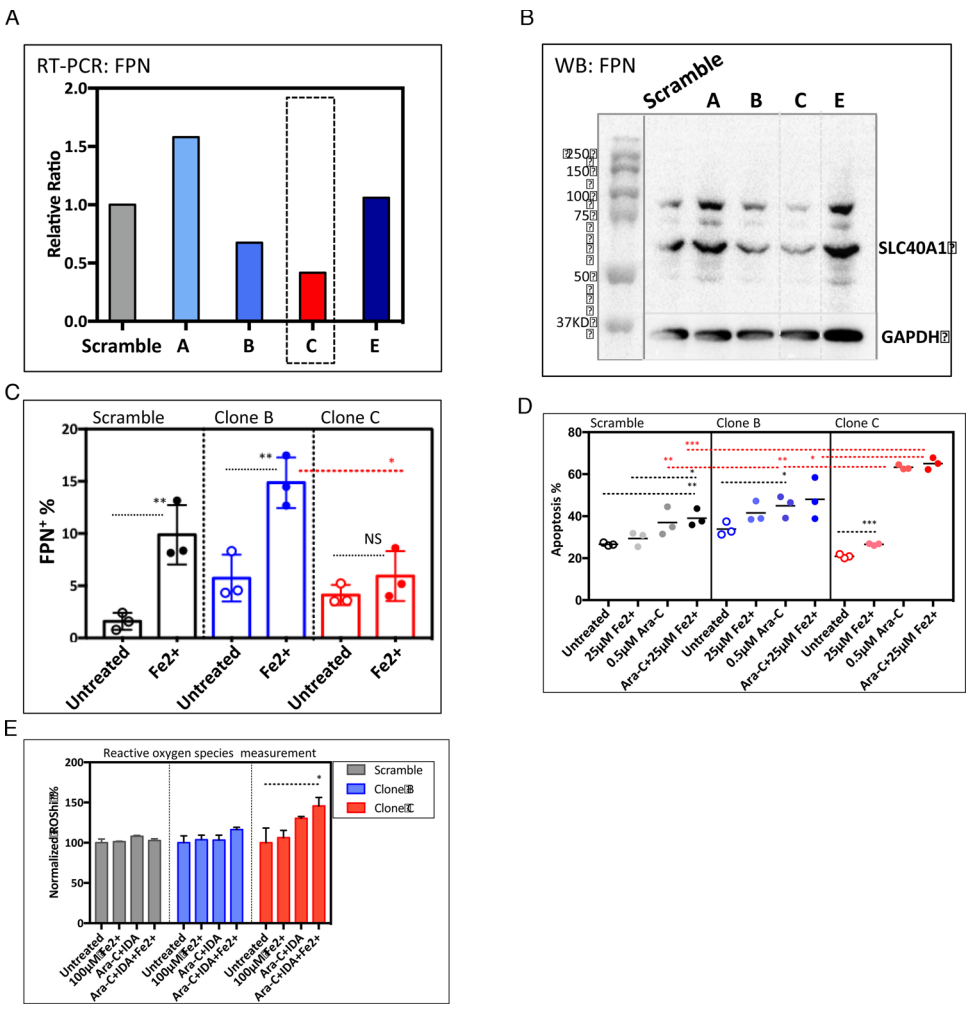


Fig. 3. shRNA knockdown of FPN in MOLM16 leads to increased chemotherapy sensitivity. (A–C) Characterization of FPN knockdown in the FPN^{high} MOLM16 line. Lentivirus gene transfer of SLC40A1 shRNA into 4 MOLM16 clones (*A-B-C-E*) and one scramble control expressing GFP. Clones' cells were sorted at high purity based on GFP expression and then subjected to analysis including RT-PCR (A), western blot (B) and flow cytometry (C) respectively. (C) Summary bar graph of FPN expression by flow cytometry (at baseline and after 2 h of 100μM Fe^{2+} treatment) is displayed for each clone (n = 3; ** < 0.005; * < 0.05). (D) Sensitivity of FPN knockdown MOLM16 to Fe^{2+} and chemotherapy. Following lentivirus gene transfer of SLC40A1 shRNA, MOLM16 were treated with 25μM Fe^{2+}, 0.5μM Ara-C or both and then detection of apoptosis using the Annexin V/DAPI assay was performed 48 h later (n = 3; * < 0.05; ** < 0.005; *** < 0.0005). (E) ROS measurements of SLC40A1 shRNA in MOLM16 cells. Normalized bar graphs are displayed from 2 clones and the scramble control samples (n = 3; * < 0.05).

pathways and properties between breast cancer and AML that could impact the role of FPN. In addition, breast cancer and other cancers are treated very differently from AML, and the differences in outcomes may be due as much to these treatment differences as to biological differences [43,44].

One potential explanation for the improved outcome to chemotherapy treatment of AMLs that express lower levels of FPN is an increased sensitivity of this subset of AML to the effects of chemotherapy. The findings that FPN^{low} AML cell lines and primary cells were more sensitive than FPN^{high} AMLs to chemotherapy alone, or chemotherapy combined with Fe^{2+}, support this contention. A possible model for the increased sensitivity to chemotherapy in the FPN^{low} AMLs is presented in Fig. 5. In this model, lower levels of FPN lead to higher levels of intracellular iron, which in turn leads to higher levels of ROS generation following oxidative stress such as exposure to chemotherapy. The higher intracellular ROS levels following chemotherapy treatment in turn leads to higher levels of apoptosis and thus improved outcomes. However, other direct and indirect effects of FPN levels and iron metabolism on AML treatment outcomes remain possible and the mechanism for the improved survival of FPN^{low} AML patients merits further study. Similarly the role of other regulators of iron homeostasis such as hepcidin and related proteins like the Poly(rc)-binding proteins (PCBPs), also warrant further study in the biology and treatment responses of AML [43,45]. In support of this, downregulation of PCBP-1 has previously been shown to have poor prognostic significance in AML [46]. While the FPN knockdown experiments in this study suggest a direct relationship between FPN and chemotherapy and/or iron sensitivity, additional experiments are warranted to further bolster the contention that there is a causal connection between FPN levels, chemotherapy sensitivity and outcomes in AML. These could include overexpressing FPN in FPN^{low} AML cell lines and primary cells and challenging them with chemotherapy and/or iron as well as measuring intracellular ferritin, iron and other iron related proteins and pathways following chemotherapy. In addition, it remains possible that other processes, either wholy or in part, are responsible for the association between FPN levels in AML cells and outcomes and these should be explored as well. It would also be interesting to study the association and role of FPN in chemotherapy resistance that arises with relapsed disease and progressive lines of therapy. A preliminary study showed no obvious differences between SLC40A1 levels at diagnosis (i.e. DeNovo AML) or relapse (see Fig. S3 in the Supplemental figures) but larger data sets and prospective studies are needed to further explore this issue.

In addition to these mechanistic considerations, our studies suggest that modulating iron homeostasis and/or FPN levels may also be useful as a treatment adjunct for AML. As one possibility, treating FPN^{low} AML patients with ferrous sulfate may potentiate chemotherapeutic effects. Developing a clinical trial of oral or i.v. iron therapy along with low dose cytarabine in older patients with FPN^{low} AMLs may be interesting, particularly as iron deficiency is relatively common among the elderly population. Similarly, agents that decrease FPN either directly or indirectly through hepcidin may also potentiate chemotherapy and other agents. Recent studies showing the therapeutic effects of knocking down FPN in a preclinical model of neuroblastoma support this

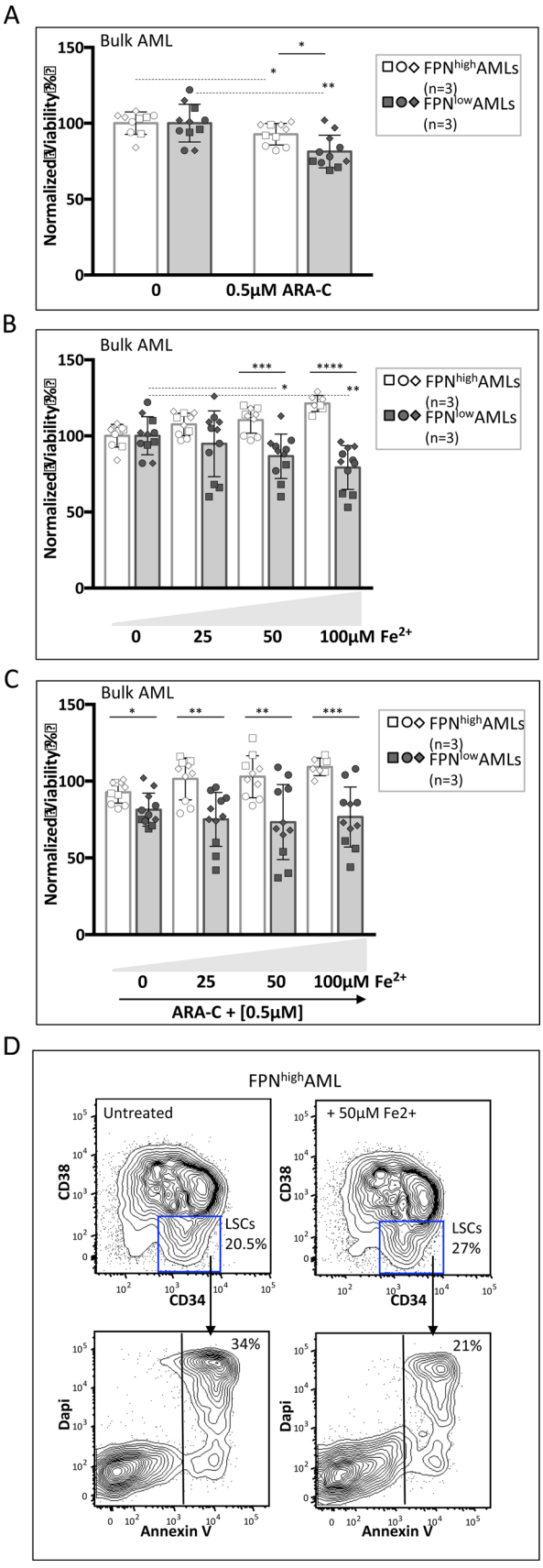
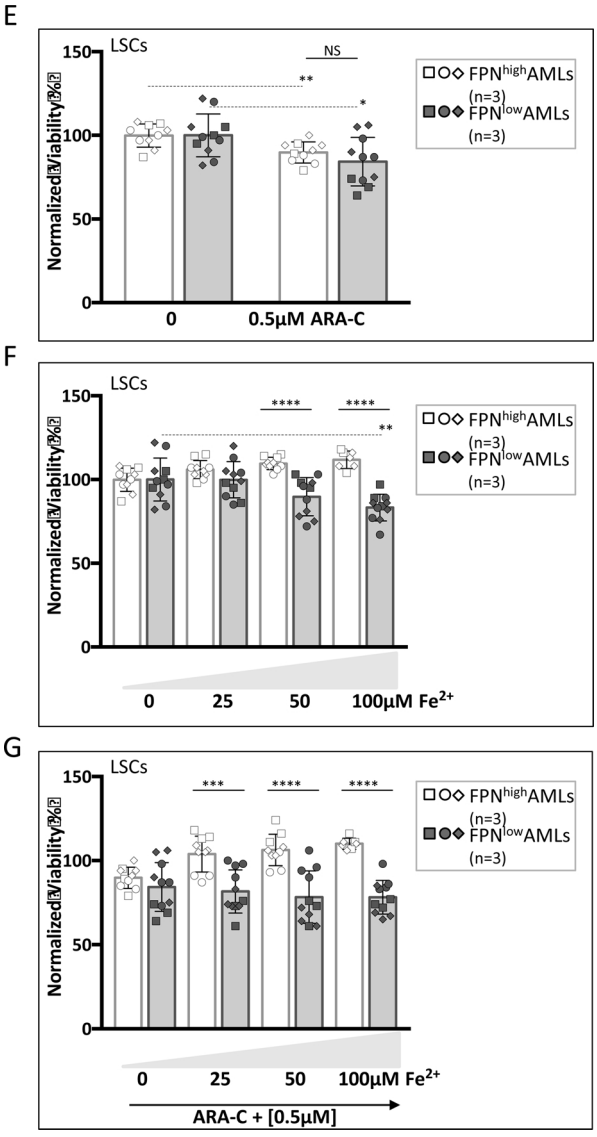


Fig. 4. Reduced levels of FPN in primary AMLs correlates with increased chemosensitivity. (A–C) Three samples of FPN^{high} and three FPN^{low} AMLs were treated with 0.50μM Ara-C plus or minus 25μM, 50μM and 100μM Fe^{2+} for 24 h. Apoptosis (Annexin V/DAPI) analysis was measured in the total AML population following treatment with Ara-C alone (A), iron alone (B) or Ara-C plus iron (C). To analyze similarly treated LSCs, the LSC subset was defined by flow cytometric as depicted in (D) for a representative FPN^{high} AML. Apoptosis within LSC gates was then determined following Ara-C alone, iron alone and Ara-C plus iron as above for the total AML population (E–G respectively). Individual values (each symbol represents a biological sample in replicates) were plotted in a column scatter graph showing mean and SD (n = 3 with 3–4 replicates per sample; * < 0.05; ** < 0.005; *** < 0.0005 and **** < 0.0001).

suggestion [47]. Conversely, in FPN^{high} AML, iron chelating agents may be useful to reverse the apparent protective effect of iron we observed in this subset of AMLs [17,23]. As another alternative, agents that induce or increase ferroptosis such as sorafenib or erastin anologues may also increase sensitivity of AML to chemotherapy [48,49].



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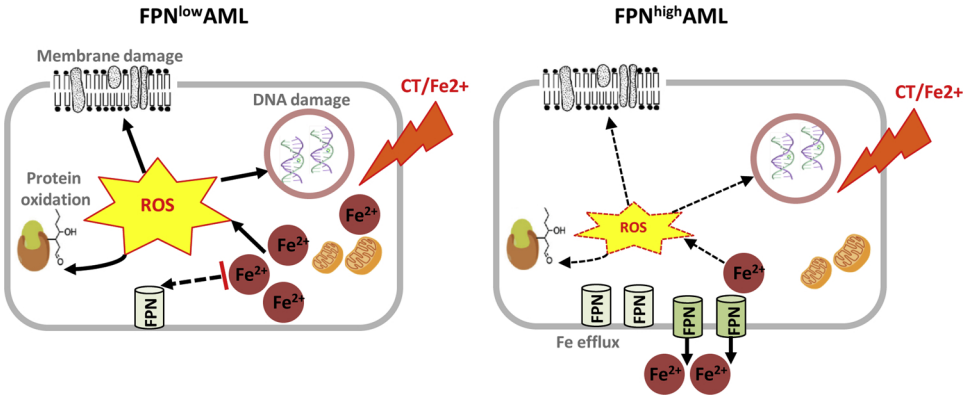

Fig. 5. Model for the role of FPN in AML and response to therapy. In this model, lower levels of FPN lead to higher levels of intracellular iron which in turn leads to higher levels of ROS generation following exposure to ROS inducing treatment agents. Higher levels of ROS in turn lead to increased cytotoxicity and better treatment outcomes. (CT = chemotherapy, FPN = ferroportin).

5. Conclusions

Low levels of FPN in AML may have important good prognostic significance. This may occur in part because of an increased sensitivity of FPN^{low} AMLs to chemotherapy. These findings may point the way to future mechanistic insights into the biology of AML and have practical implications for the treatment of AML.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2019.02.011.

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