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MYELODYSPLASTIC NEOPLASM

Myelodysplastic neoplasm-associated U2AF1 mutations induce host defense defects by compromising neutrophil chemotaxis

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Myelodysplastic neoplasm (MDS) is a hematopoietic stem cell disorder that may evolve into acute myeloid leukemia. Fatal infection is among the most common cause of death in MDS patients, likely due to myeloid cell cytopenia and dysfunction in these patients. Mutations in genes that encode components of the spliceosome represent the most common class of somatically acquired mutations in MDS patients. To determine the molecular underpinnings of the host defense defects in MDS patients, we investigated the MDS-associated spliceosome mutation U2AF1-S34F using a transgenic mouse model that expresses this mutant gene. We found that U2AF1-S34F causes a profound host defense defect in these mice, likely by inducing a significant neutrophil chemotaxis defect. Studies in human neutrophils suggest that this effect of U2AF1-S34F likely extends to MDS patients as well. RNA-seq analysis suggests that the expression of multiple genes that mediate cell migration are affected by this spliceosome mutation and therefore are likely drivers of this neutrophil dysfunction.

INTRODUCTION

Myelodysplastic neoplasm (MDS) is a hematopoietic stem cell disorder characterized by defects in myeloid cell differentiation, production of dysplastic blood cells, and risk of evolution to acute myeloid leukemia (AML) [1]. Approximately 10,000–40,000 patients are newly diagnosed with MDS in the United States annually [2–4]. MDS occurs primarily in elderly individuals [5] and, like AML, has a poor prognosis, with only a 35% 3-year survival rate [6]. In MDS patients, defects in myeloid stem cell differentiation can induce anemia due to erythrocyte defects, hemorrhage due to platelet disorders, and increased infection risk due to neutropenia or neutrophil dysfunction. Fatal infection and infectious complications are among the most common cause of death in MDS patients, accounting for between 18% and 53% of all deaths in MDS patients [7–10]. Gram negative and Gram positive bacterial infections are fairly common in MDS patients; while viral and fungal infections also occur, these are rarer.

Many patients with MDS are neutropenic, which accounts for some but not all of the increased infection risk in these patients [11–16]. Even when neutrophils are produced in patients with MDS, these neutrophils exhibit a range of functional defects, and this neutrophil dysfunction also likely accounts for the increased susceptibility to infection in patients with MDS [11–16].

What drives this neutrophil dysfunction and the resulting increased infection risk in MDS patients is still unclear. Recent next-generation sequencing studies have identified many recurrent mutations in MDS patients [17]. The most common class of genes that are somatically mutated in MDS are those that regulate pre-mRNA splicing, with roughly half of all MDS patients having a mutation in the splicing machinery [18]. The removal of introns from pre-mRNA is driven by the spliceosome, a large ribonucleo-protein complex [19]. Three spliceosome genes, U2AF1, SF3B1, and SRSF2 are frequently somatically mutated in MDS patients [20–22]. These spliceosome mutations act as gain of function or neomorphic mutations [20–22] that induce changes in gene expression and pre-mRNA splicing on a genomic scale [20–22]. Mice engineered to express these MDS-associated spliceosome gene mutations exhibit hematopoietic defects [23, 24] suggesting that these spliceosome mutations are driver mutations that contribute to disease pathogenesis.

In particular, mice engineered to express the common U2AF1-S34F mutation exhibit hematopoietic defects [25, 26]. Expression of human U2AF1-S34F in mice induced cytopenias in B cells and monocytes and affected common myeloid progenitor production [25]. Inhibition of U2AF1 or other MDS-associated splicesome mutations weakens inflammation by altering splicing in TLR signaling pathways [27, 28]. In contrast, the neomorphic

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MDS-associated U2AF1-S34F mutation and other spliceosome mutations enhance pro-inflammatory signaling pathways [28–33]. The effects of altered innate immune signaling induced by MDS-associated spliceosome mutations on host defense have not been investigated previously even though MDS patients exhibit substantial immunodeficiency.

Here we investigate the effect of the MDS-associated U2AF1-S34F mutation on host defense using mice engineered to express human U2AF1-S34F and using peripheral blood samples from patients with MDS. We find that mice expressing U2AF1-S34F are profoundly immunodeficient, rapidly succumbing to *E. coli* infection. This is likely due to neutrophil chemotaxis defects induced by the U2AF1-S34F mutation. Multiple gene expression changes in neutrophils from U2AF1-S34F mice may drive this neutrophil dysfunction. Studies using neutrophils from MDS patients suggest that U2AF1 mutation weakens neutrophil chemotaxis ability in humans as well.

MATERIALS AND METHODS
U2AF1 mice
All mouse studies were approved by the National Jewish Health Institutional Animal Care and Use Committee with the protocol number AS2801-05-24. Mice engineered to inducibly express wild type U2AF1 (U2AF1-wt, rtTA) or the U2AF1-S34F mutant variant (U2AF1-S34F, rtTA) have been described [25]. These mice express human U2AF1 transgenes; mouse and human U2AF1 differ in only one amino acid and are >99% identical. Transgenic mice were bred with C57BL/6J-129 wild type mice to generate heterozygous U2AF1-S34F and U2AF1-wt mice following the breeding scheme outlined in [25]. To induce U2AF1 transgene expression, 8–12 week old male and female mice were injected via intraperitoneal injection with 25 mg/kg 9-tert-Butyl Doxycyline (9-TB) in PBS daily for four consecutive days. All experiments were then initiated on the fifth day.

See online Supplementary Methods for all other Methods.

RESULTS
Mice expressing U2AF1-S34F are susceptible to infection
To assess the effect of the MDS-associated U2AF1-S34F mutation on host defense, we analyzed mice engineered to express U2AF1-S34F using a rtTA doxycycline-inducible system (rtTA, U2AF1-S34F) compared to control mice engineered to express a wild type U2AF1 transgene (rtTA, U2AF1-wt) [25]. Because doxycycline has antimicrobial properties, we induced transgene expression using a doxycycline analog (9-tert-Butyl Doxycycline HCl or 9-TB) that was previously reported to not exhibit antimicrobial activity [34], and which we confirmed did not kill *E. coli* (Supplementary Fig. 1).

Mice heterozygous for the U2AF1-S34F transgene and control mice heterozygous for U2AF1-wt were injected daily for four days via intraperitoneal injection with 9-TB, which induced moderate overexpression of the U2AF1 transgenes similar to expression induced by including doxycycline in the mouse chow [28]. One day after the last 9-TB injection, mice were injected via intraperitoneal injection with 10^8 CFU *E. coli* strain H9049, a human patient isolate of *E. coli*. Four or eight hours after infection, the mice were humanely euthanized, and viable bacteria in peritoneal lavage, blood, liver, and spleen were assessed by counting colony forming units (CFUs) (Fig. 1A–H). In all cases, significantly more viable bacteria were recovered from the mice expressing the U2AF1-S34F transgene compared to mice expressing U2AF1-wt.

The rtTA, U2AF1-S34F mice express the U2AF1 transgene in most tissues. To confirm that U2AF1-S34F was inducing host defense defects due to its expression in the hematopoietic system, we lethally irradiated wild type recipient mice and then transferred marrow from either rtTA, U2AF1-S34F mice or rtTA, U2AF1-wt mice into the irradiated recipients. Six weeks after marrow transfer, the recipient mice were injected daily for four days with 9-TB, and then the mice were infected with *E. coli* strain H9049. Mice expressing U2AF1-S34F in the hematopoietic system,

but not mice expressing U2AF1-wt, exhibited higher viable bacterial counts in peritoneal lavage after infection (Fig. 1I).

To determine the effect of U2AF1-S34F on host defense, we also monitored survival of U2AF1-S34F mice and U2AF1-wt control mice after infection with *E. coli*. While almost all wild type mice were able to survive the infection, U2AF1-S34F mice exhibited significant morbidity, with almost all mice succumbing to infection within 24 h (Fig. 1J). Thus, mice expressing U2AF1-S34F exhibited significant host defense defects as assessed by bacterial CFU counts and mouse morbidity.

Neutrophils in mice expressing U2AF1-S34F fail to migrate to the infection site
To determine why U2AF1-S34F mice succumbed to *E. coli* infection, we monitored the production of the pro-inflammatory cytokines TNFα and IL-6 in the peritoneum and blood after peritoneal *E. coli* infection. Mice expressing U2AF1-S34F produced as much or more pro-inflammatory cytokines in their peritoneum and blood than did mice expressing U2AF1-wt (Supplementary Fig. 2A–H), indicating that despite their host defense deficiency, U2AF1-S34F mice were still capable of detecting the presence of the pathogen.

We also examined recruitment of inflammatory cells to the peritoneum after peritoneal *E. coli* infection. Intraperitoneal (I.P.) infection with *E. coli* induced a robust inflammatory cell infiltration composed of neutrophils and monocytes/macrophages in mice expressing U2AF1-wt. In contrast, neutrophils in mice expressing U2AF1-S34F largely failed to migrate to the site of infection (Fig. 2A, B). Bone marrow chimera studies performed as described above confirmed that this neutrophil chemotaxis defect was caused by U2AF1-S34F expression in the hematopoietic system. I.P. *E. coli* infection induced robust inflammatory cell infiltration into the peritoneum in mice receiving wild type U2AF1 marrow; in contrast, mice that received U2AF1-S34F marrow had a significant defect in neutrophil recruitment to the peritoneum (Fig. 2C, D).

Neutrophils from U2AF1-S34F mice exhibit an intrinsic migration defect
There are several possibilities that could explain why neutrophils fail to migrate to the infection site in U2AF1-S34F mice following peritoneal infection. These include: (1) lack of neutrophil production in the U2AF1-S34F mice, (2) lack of signals needed for neutrophil recruitment in U2AF1-S34F mice, or (3) an intrinsic migration defect in neutrophils in U2AF1-S34F mice. We tested each of these three possibilities in turn as outlined below.

First, we quantitated the basal number of neutrophils present in uninfected mice, and found that neutrophil counts were similar in peritoneum and blood in mice expressing U2AF1-S34F or U2AF1-wt (Fig. 3A, B). In the bone marrow, while neutrophils were present, there was a significant reduction in the total number of neutrophils in the U2AF1-S34F mice (Fig. 3C) and a comparable decrease in the number of mature neutrophils in these mice (Fig. 3D). This decrease in total neutrophil counts in U2AF1-S34F mice is likely due to a decrease in overall marrow cellularity in these mice, as the percentage of neutrophils in the marrow in the U2AF1-S34F mice was not significantly different than in U2AF1-wt mice (37.9 ± 2.1 WT vs 32.5 ± 2.1 S34F, mean ± SEM, $p = 0.08$). As reported previously [25], monocyte counts were significantly reduced in the U2AF1-S34F mice (75 ± 21 WT vs 13 ± 6 S34F, cells/ml blood × 10^3, $p = 0.03$). Because blood and marrow neutrophils could both contribute to peritoneal infection-mediated neutrophil recruitment, it is possible that the diminished neutrophil counts in the marrow contribute, at least in part, to the neutrophil recruitment defect present in U2AF1-S34F mice.

We next examined production of factors required for neutrophil migration, including KC, a chemokine involved in neutrophil recruitment, and GM-CSF and G-CSF, which are required for neutrophil maturation and emigration from the bone marrow [35, 36]. Production of all these factors was similar to or higher in
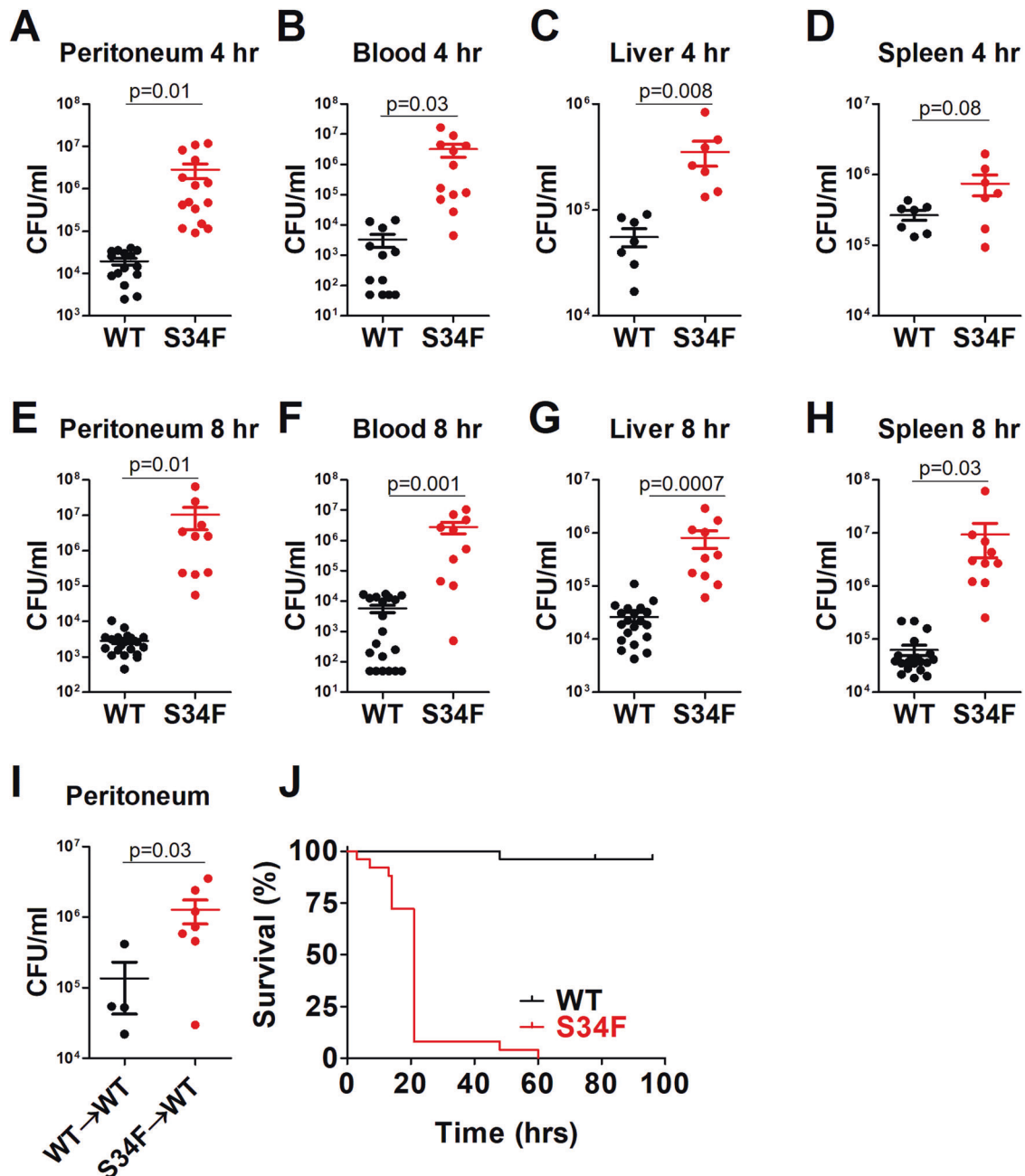
Fig. 1 Mice expressing U2AF1-S34F are unable to control an *E. coli* infection. U2AF1-S34F, rtTA mice and control U2AF1-wt, rtTA mice were injected daily for four days with 9-TB via intraperitoneal injection. The mice were subsequently infected with 10^8 CFU of *E. coli* strain H9049 via intraperitoneal injection. Four (**A–D**) or eight (**E–H**) hours later, the mice were humanely euthanized, and viable bacteria were quantitated by counting colony forming units (CFUs) in peritoneal lavage, blood, liver, and spleen. In all cases, U2AF1-S34F mice had significantly higher CFU counts than the control U2AF1-wt mice. CFU counts in some blood samples from U2AF1-wt mice were not detected on the lowest dilution plate, and are graphed as the maximum theoretical CFU count. **I** To confirm that hematopoietic expression of U2AF1-S34F was inducing this host defense defect, bone marrow in wild type recipient mice was ablated with lethal irradiation, and donor marrow from either U2AF1-S34F, rtTA mice or U2AF1-wt, rtTA mice was implanted in the recipients. Six weeks later, transgene expression was induced by four daily injections of 9-TB. Subsequently, the mice were infected via intraperitoneal injection with 10^8 CFU of *E. coli* strain H9049; CFU counts were determined 4 h later. **J** U2AF1-S34F, rtTA and U2AF1-wt, rtTA mice were injected with 9-TB daily for 4 days and were then infected via intraperitoneal injection with 10^8 CFU of *E. coli* strain H9049. Mouse morbidity was then monitored. $N = 26$ in each group, $p < 0.0001$. In this figure and all subsequent figures, WT (black dots/lines) indicate U2AF1-wt, rtTA mice; S34F (red dots/lines) indicates U2AF1-S34F mice.

mice expressing U2AF1-S34F compared to mice expressing U2AF1-wt following I.P. *E. coli* infection (Fig. 4A–H), indicating that extrinsic factors that drive neutrophil migration are present in U2AF1-S34F mice. This suggests that the neutrophil chemotaxis defect observed in U2AF1-S34F mice might be due to intrinsic defects in the neutrophils themselves.

To test if neutrophils in U2AF1-S34F mice were intrinsically deficient in migration, we injected the chemokine KC into the peritoneum of mice expressing U2AF1-S34F or U2AF1-wt. Even when the chemokine was introduced directly into these mice, U2AF1-S34F neutrophils failed to migrate into the peritoneum (Fig. 4I). In contrast, KC induced robust neutrophil recruitment in
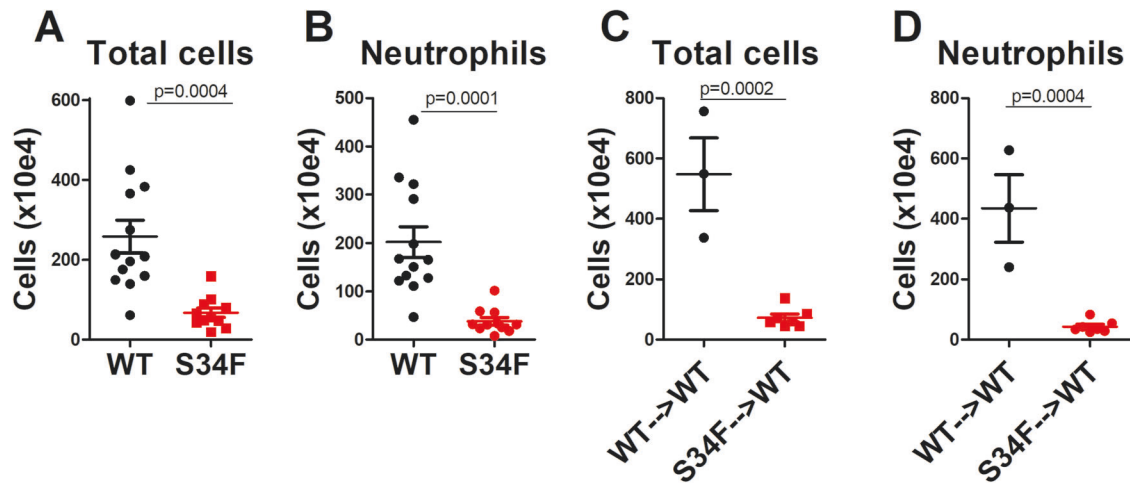
Fig. 2 Neutrophils fail to migrate to the infection site in U2AF1-S34F mice. A, B U2AF1-S34F, rtTA mice and U2AF1-wt, rtTA mice were injected daily with 9-TB for 4 days and then were infected via intraperitoneal injection with 10^8 CFU *E. coli* strain H9049. Six hours later, total cells in peritoneal lavage were counted with a hemocytometer. Cell differentials were analyzed by staining cytospin slides; total neutrophil counts were calculated by multiplying the fraction of total cell that were neutrophils. The remaining cells were all monocytes/macrophages. **C, D** Bone marrow was ablated in wild type recipient mice, and then marrow from either U2AF1-S34F, rtTA or U2AF1-wt, rtTA donor mice was injected via tail vein injection. Six weeks after marrow engraftment, the mice were injected daily for four days with 9-TB and were then infected with *E. coli* via intraperitoneal injection. Four hours after infection, total cell counts and neutrophil counts in the peritoneum were analyzed as described in (**A**) and (**B**).
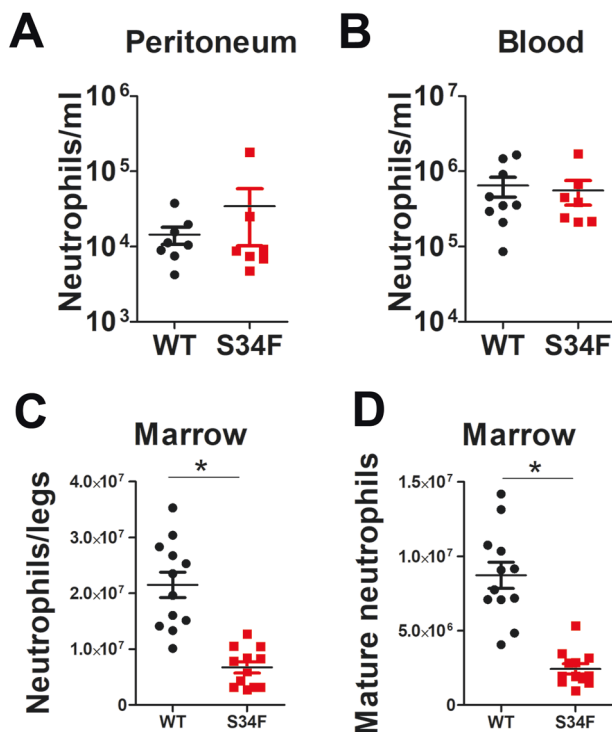

Fig. 3 Neutrophils are present in U2AF1-S34F mice. U2AF1-S34F, rtTA and U2AF1-wt, rtTA mice were injected daily for 4 days with 9-TB. The mice were subsequently euthanized, and neutrophil counts in peritoneal lavage, blood, and bone marrow were assessed by flow cytometry. Neutrophil counts in lavage and blood are reported as total neutrophils per ml lavage fluid or blood, respectively. Neutrophils in bone marrow are reported as total neutrophils or total mature neutrophils per mouse, quantifying four leg bones (femurs and tibias) in each mouse.

U2AF1-wt mice (Fig. 4I). Because both blood and bone marrow neutrophils could contribute to neutrophil recruitment, we also directly monitored neutrophil emigration from the bone marrow. When mice were injected intravenously (I.V.) with G-CSF,

neutrophils emigrated from the bone marrow into the blood in mice expressing U2AF1-wt but not in mice expressing U2AF1-S34F (Fig. 4J). All these data suggest that neutrophils from U2AF1-S34F mice have an intrinsic migration defect.

To confirm that the neutrophil migration defect in U2AF1-S34F mice is due to an intrinsic neutrophil defect, we purified neutrophils from mouse bone marrow and monitored chemotaxis towards KC in an ex vivo cell culture system. Isolated neutrophils from U2AF1-S34F mice migrated less efficiently towards KC than did neutrophils from U2AF1-wt mice (Fig. 5A). Even though neutrophils from U2AF1-S34F mice were defective in migration, they were not defective in all neutrophil functions. For example, neutrophils isolated from U2AF1-S34F mice phagocytosed *E. coli* particles and produced reactive oxygen species similarly to neutrophils from U2AF1-wt mice (Fig. 5B, C).

Neutrophils from MDS patients carrying U2AF1 mutations exhibit a chemotaxis defect

To determine if the neutrophil chemotaxis defect present in mice expressing U2AF1-S34F was also present in patients harboring this mutation, we isolated peripheral blood neutrophils from patients with MDS and from age and sex matched healthy volunteers (demographic information in Supplementary Tables 1 and 2, respectively). Neutrophil migration in a transwell cell culture system was monitored in either the absence of chemokines or in the presence of the chemotactic factor IL-8. IL-8 was able to significantly increase the migration of neutrophils from healthy donors (Fig. 6). In contrast, IL-8 did not significantly increase the migration of neutrophils isolated from MDS patients (Fig. 6). This migration defect was present in neutrophils from MDS patients that did not have a mutation in spliceosome genes and in neutrophils from patients that had mutations in either the U2AF1 or SF3B1 spliceosome genes (Fig. 6). Thus, MDS patient neutrophils exhibited a migration defect in all genotypes tested. These data suggest that multiple MDS-associated spliceosome mutations are capable of inducing neutrophil chemotaxis defects.

Neutrophils from U2AF1-S34F mice have multiple defects in pathways controlling chemotaxis

To determine why neutrophils from U2AF1-S34F mice exhibit a cell migration defect, we first monitored production of the
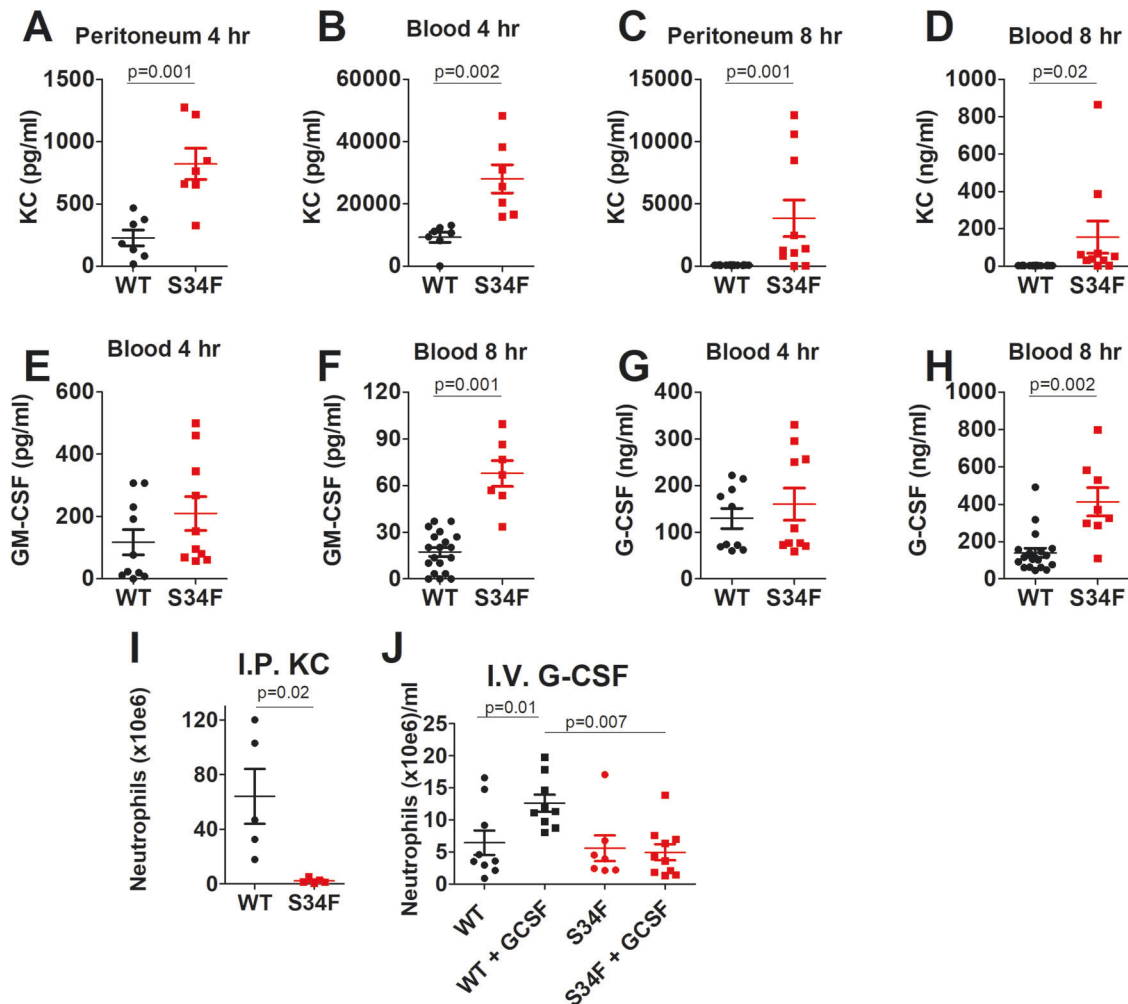
Fig. 4 Neutrophils in U2AF1-S34F mice have an intrinsic cell migration defect. U2AF1-S34F, rtTA or U2AF1-wt, rtTA mice were injected daily for four days with 9-TB. **A–H** The mice were then infected with 10^8 CFU *E. coli* via intraperitoneal injection. At the indicated times, the mice were humanely euthanized, and the indicated cytokines and chemokines were quantitated by ELISA in peritoneal lavage or blood. If no P value is listed in a particular panel, then the U2AF1-S34F and U2AF1-wt readings were not statistically different in that case. **I** The mice were injected via intraperitoneal injection with 500 ng KC. Four hours later, the number of infiltrating neutrophils was quantitated in peritoneal lavage. **J** The mice were injected with 3.12 µg G-CSF (or not as a control); 4 h later, the mice were humanely euthanized, and the number of neutrophils that emigrated into the blood were quantitated.

chemokine receptor CXCR2. Neutrophil migration is regulated by CXCR2, which responds to chemokines including IL-8 and GRO-α [37]. Surface levels of CXCR2 on neutrophils expressing U2AF1-S34F were largely unchanged as assessed by flow cytometry (Supplementary Fig. 3A). Likewise, ELISA analysis of CXCR2 levels in neutrophils from U2AF1-S34F mice identified a statistically significant decrease in CXCR2 levels in U2AF1-S34F mice; however, this decrease was fairly modest (Supplementary Fig. 3B), and its biological relevance is therefore unclear.

To determine why U2AF1-S34F neutrophils are defective in chemotaxis, we used RNA-seq to monitor the full complement of gene expression and mRNA splicing changes induced by U2AF1-S34F in neutrophils. Bone marrow neutrophils were isolated from mice expressing either U2AF1-S34F or U2AF1-wt, either in mice not infected or in mice infected with *E. coli* H9049 for 4 h. RNA was prepared from isolated neutrophils, and RNA-seq was performed. Principal component demonstrated that both mouse genotype and the presence or absence of infection altered gene expression in these neutrophils (Supplementary Fig. 4).

The presence of the U2AF1-S34F mutation (comparing neutrophils from U2AF1-S34F mice to neutrophils from U2AF1-wt mice) induced substantial changes in gene expression (Fig. 7A, B

and Supplementary Table 3) and pre-mRNA splicing (Fig. 7C, Supplementary Table 4) in either the absence or presence of infection. These mRNA splicing changes included all classes of alternative splicing events, although approximately half of them were exon skipping events (Fig. 7C). We used Gene ontology (GO) analysis as a non-biased approach to identify pathways affected by U2AF1-S34F in neutrophils, comparing U2AF1-S34F to U2AF1-wt. Interestingly, GO-pathway analysis of mRNA splicing changes induced by U2AF1-S34F mice indicated that many RNA processing pathways were affected by the U2AF1 mutation (Supplementary Table 5). This observation is consistent with the observed effects of MDS-associated spliceosome mutations on alternative splicing in other cell types [32, 38, 39].

We also monitored the effects of U2AF1-S34F (compared to U2AF1-wt) on gene expression in neutrophils. In the absence or presence of infection, U2AF1-S34F increased the expression of 125 or 267 genes, respectively (Fig. 7A). More than half of the biological pathways upregulated in U2AF1-S34F neutrophils were immune-related pathways (Fig. 7D, Supplementary Tables 6 and 7). Among the GO categories over-represented in genes upregulated by U2AF1-S34F were "cellular response to interferon-alpha" and "cellular response to interferon-beta." These data
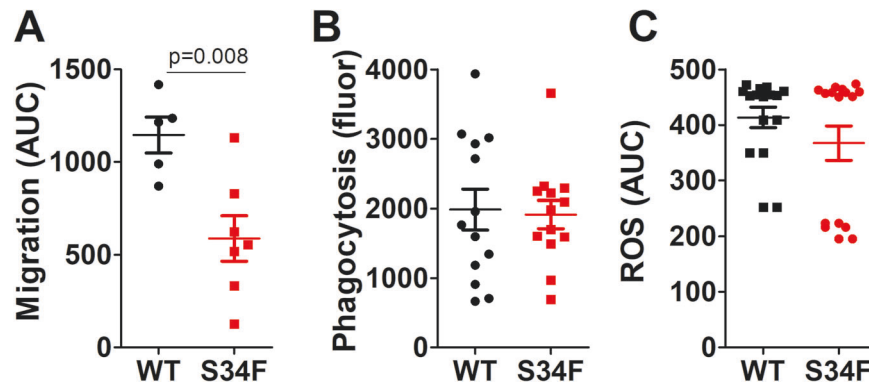
Fig. 5 Neutrophils from U2AF1-S34F mice fail to migrate properly. U2AF1-S34F, rtTA or U2AF1-wt, rtTA mice were injected daily for four days with 9-TB. Bone marrow neutrophils were isolated and subjected to analysis ex vivo. **A** Neutrophils were fluorescently labeled, and neutrophil migration towards KC was quantified over the course of 90 min. Data are graphed in arbitrary cumulative fluorescence units (i.e., area under the curve, AUC). **B** Neutrophil uptake of fluorescently labeled *E. coli* particles over the course of 1 h was quantitated. Data in arbitrary fluorescence units. **C** Production of reaction oxygen species (ROS) was assessed by monitoring fluorescence of the ROS-reactive CM-H2DCFDA indicator for 2 h. Data in arbitrary cumulative fluorescence units (i.e., area under the curve, AUC). Data in graph panels that do not list a *p* value were not significantly different.
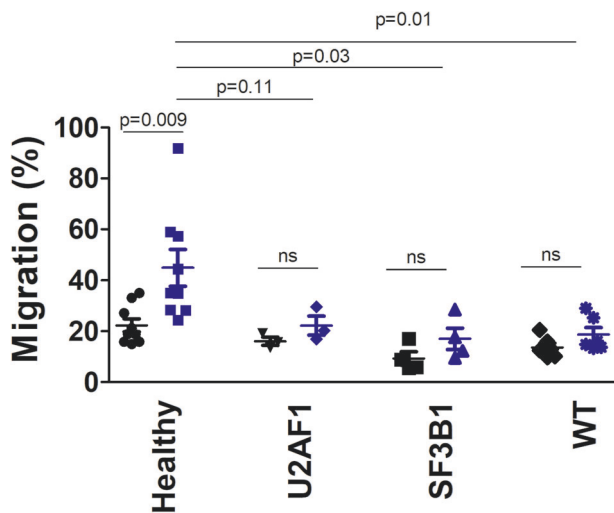

Fig. 6 Neutrophils from MDS patients fail to migrate properly. Peripheral blood neutrophils were isolated from healthy blood donors and from patients with MDS. The MDS patient neutrophils were stratified by those harboring mutations in U2AF1, SF3B1, or those lacking any spliceosome mutation (WT). Neutrophils were fluorescently labeled, and neutrophil migration across a transwell in either the absence of stimulation (black dots) or the presence of 25 ng/ml IL-8 stimulation (blue dots) was quantified over the course of 90 min.

suggest that interferon signaling may be altered in neutrophils from U2AF1-S34F mice.

Pathway analysis of genes that were down-regulated by U2AF1-S34F in the absence or presence of infection also identified changes in immune-relevant pathways (Fig. 7B, Supplementary Tables 8 and 9). Notably, pathways relevant to cell migration and chemotaxis were down-regulated in neutrophils from U2AF1-S34F mice (Fig. 7E and Supplementary Tables 8 and 9), consistent with the chemotaxis defect observed in these mice. These migration-relevant pathways included but were not limited to "positive regulation of monocyte chemotaxis," "lymphocyte migration," and "mononuclear cell migration." Other relevant pathways that were downregulated include "chemokine-mediated signaling pathway" and "cellular response to chemokine." Finally, "cellular extravasation," which could also affect neutrophil migration to the infection site, was altered [40–42]. Altogether, decreased expression in 18

different genes known to affect cell migration and chemotaxis were identified in this pathway analysis (Supplementary Fig. 5, Supplementary Table 10). Decreased expression of these 18 genes drove the pathway-level changes in cell migration observed (Fig. 7E). This suggests that decreased expression of these 18 genes could contribute to the neutrophil chemotaxis defect observed in U2AF1-S34F mice.

To confirm that gene expression in cell migration pathways was decreased in neutrophils from U2AF1-S34F mice, we also used a second pathway analysis platform, Ingenuity Pathway Analysis (IPA). IPA analysis of gene expression changes induced by U2AF1-S34F confirmed that cell migration pathways were downregulated in U2AF1-S34F neutrophils. Again, multiple gene expression changes contributed to the predicted decrease in expression of genes in these migration pathways (Supplementary Table 11). This analysis also suggested that downregulated chemotaxis-relevant gene expression could contribute to the cell migration defect in U2AF1-S34F neutrophils.

To determine if any of these genes might also be contributing to the chemotaxis defect present in neutrophils from patients with MDS, we used qPCR to examine expression of the human homologs of these genes. 12 of the 13 genes with the largest decrease in expression in U2AF1-S34F mouse neutrophils had human orthologs (Supplementary Table 10). We used qPCR to examine expression of these 12 genes in human neutrophils; expression of 8 of these 12 genes was above background levels (Fig. 8). Compared to age and sex matched neutrophils from healthy donors, neutrophils from patients with U2AF1 mutations exhibited decreased expression of several of these genes, although only two (AIF1 and PADI2) approached or reached statistical significance, probably because of the small size of this human patient cohort (Fig. 8). It is possible that U2AF1-mutation induced changes in expression of these genes contribute to the chemotaxis defect present in neutrophils from MDS patients.

DISCUSSION

MDS patients are known to have heightened susceptibility to infection, and infection is among the most common causes of death in these patients. Some MDS patients have significant neutropenia, which contributes to this host defense defect. Even in patients that have normal neutrophil counts, significant deficits in neutrophil function have been reported. For example, neutrophils from some patients are reported to exhibit a weakened ability to phagocytose bacteria [11, 13, 16], decreased
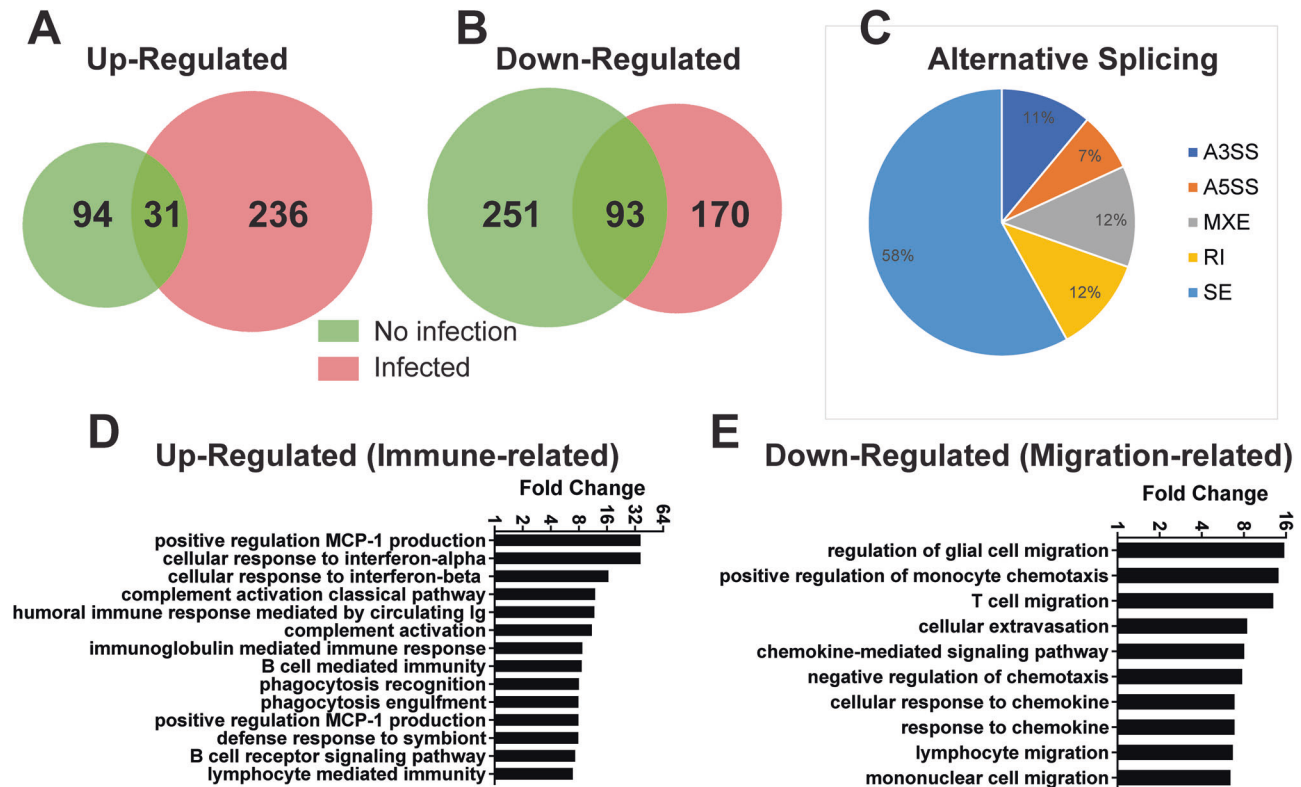
Fig. 7 U2AF1-S34F induces changes in gene expression and alternative mRNA splicing in mouse neutrophils. Neutrophils were collected from mice expressing either U2AF1-S34F or control mice expressing U2AF1-wt, either in the absence of infection or in mice infected I.P. with *E. coli* strain H9049 for 4 h. RNA was isolated and gene expression and mRNA splicing were monitored using RNA-seq. Depicted are the number of genes that were up-regulated (**A**) or down-regulated (**B**) in neutrophils from U2AF1-S34F mice compared to neutrophils from U2AF1-wt mice in the absence (green) or presence (pink) of *E. coli* infection. **C** Alternative mRNA splicing events were identified using rMATs. The pie chart indicates the frequency of different alternative splicing events identified comparing neutrophils from U2AF1-S34F to U2AF1-wt mice (N = 8515 alternative splicing events in 3117 different genes). A3SS = alternative 3' slice site used. A5SS = alternative 5' splice site used. MXE = mutually exclusive exon usage. RI = retained intron. SE=skipped exon. **D** GO Pathway analysis was performed to identify pathways that were up-regulated in neutrophils from U2AF1-S34F mice compared to U2AF1-wt mice in the absence of infection. Fold change indicates the fold enrichment of that pathway relative to control. Pathways that affected the immune system were manually extracted in (**D**); the complete set of pathways that were up-regulated are listed in Supplementary Table 6. **E** GO Pathway analysis was performed to identify pathways that were down-regulated in neutrophils from U2AF1-S34F mice compared to U2AF1-wt mice in the absence of infection. Fold change indicates the fold enrichment of that pathway relative to control. Pathways that affected cell migration were manually extracted in (**E**); the complete set of pathways that were down-regulated are listed in Supplementary Table 8.

production of important antimicrobial compounds such as elastase, myeloperoxidase, and superoxide [11–13, 43–46], and decreased production of neutrophil extracellular traps [47]. These and other defects likely contribute to the decreased bactericidal capacity of neutrophils from MDS patients [13, 16, 48]. Moreover, neutrophils from MDS patients also exhibit defects in migration towards chemotactic signals [11, 14–16, 44, 49–51]. While there are reports that some of these functional defects correlate with disease status, how mutation status in these patients impacts neutrophil function, and relatedly, what are the molecular drivers of this neutrophil dysfunction has not been addressed previously.

In the current study, we found that mice engineered to express U2AF1-S34F but not wild type U2AF1 exhibit a profound immunodeficiency, likely due to neutrophil chemotaxis defects induced by this MDS-associated spliceosome mutation. U2AF1-S34F mice were unable to control a peritoneal *E. coli* infection, with recoverable CFU counts comparable to what was instilled into these mice. In contrast, wild type mice were able to control this infection very rapidly. Despite this profound immunodeficiency, U2AF1-S34F mice were able to recognize the presence of the infection, as the U2AF1-S34F mice produced pro-inflammatory cytokines as well as factors that drive neutrophil migration and bone marrow emigration. Importantly, in U2AF1-S34F mice, neutrophils failed to migrate to the infection site, suggesting that

neutrophils from U2AF1-S34F mice were chemotaxis deficient. The decreased number of bone marrow neutrophils in U2AF1-S34F mice may contribute, at least in part, to this defect. However, neutrophils were present at levels similar to wild type in blood in the U2AF1-S34F mice, and these cells failed to migrate to the infection site, suggesting an intrinsic neutrophil migration defect. This was confirmed by monitoring neutrophil chemotaxis towards KC both in vivo and ex vivo. The neutrophils that were present in bone marrow in U2AF1-S34F mice failed to emigrate into the blood after I.V. G-CSF treatment, also consistent with an intrinsic neutrophil functional defect in these mice. Finally, studies using neutrophils from MDS patients and healthy control subjects suggested that neutrophils from MDS patients, regardless of genotype and including U2AF1-S34F mutations, were also intrinsically chemotaxis defective. These data imply that multiple MDS-associated mutations including mutations in spliceosome genes can induce neutrophil chemotaxis defects. Importantly, our mouse studies demonstrate that the MDS-associated U2AF1-S34F mutation is sufficient to induce this defect.

How does the MDS-associated U2AF1-S34F mutation impact neutrophil chemotaxis? Our studies indicated that there is at most a fairly limited decrease in levels of the chemokine receptor CXCR2 in U2AF1-S34F mice, suggesting that U2AF1-S34F likely impacts other downstream cell migration factors. This observation is in
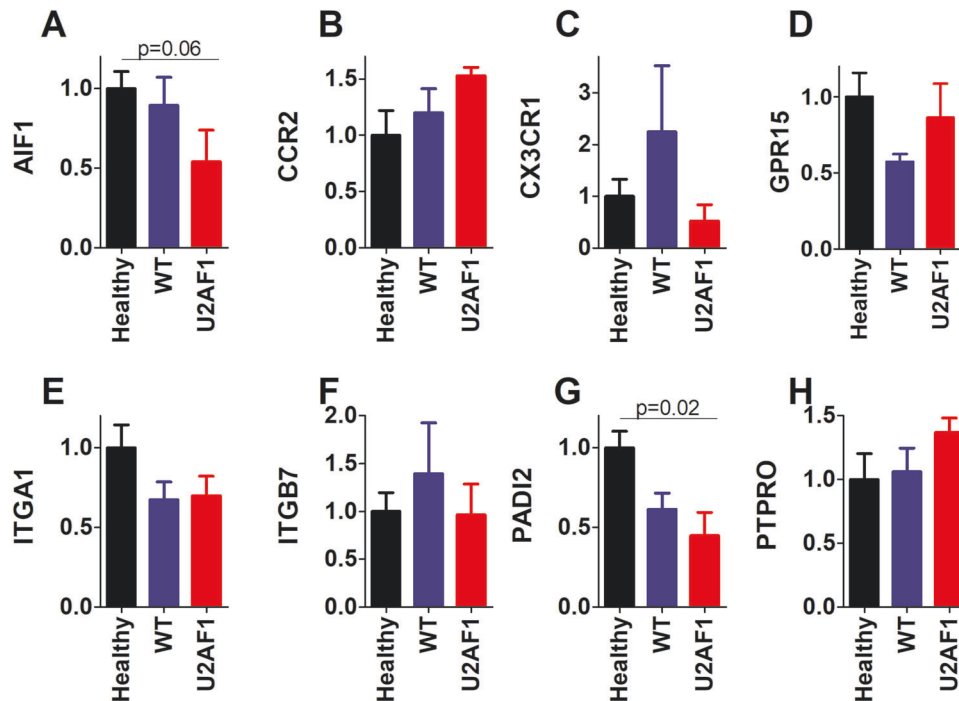
Fig. 8 Expression of genes that regulate cell migration in neutrophils from MDS patients. Neutrophils were isolated from the peripheral blood of healthy blood donors (healthy, black bars) or MDS patients without any spliceosome mutations (WT, blue bars) or MDS patients with a U2AF1 mutation (U2AF1, red bars). Gene expression of the indicated genes was monitored by qPCR, with gene expression normalized so that expression in the healthy neutrophils was set to 1. *P* values that were statistically significant are indicated; lack of a *P* value indicates that comparison was not significantly different compared to control. $N = $ (Healthy = 11, WT MDS = 5, U2AF1 MDS = 3).

agreement with a study of the migration of human neutrophils from MDS patients, in which it was observed that the neutrophil migration rate in these patients did not correlate with chemokine receptor levels [49]. This result also implicates downstream mediators as responsible for the neutrophil migration deficit present in MDS patients.

We used RNA-seq to identify changes in gene expression and pre-mRNA splicing in neutrophils that could drive this U2AF1-S34F-induced chemotaxis defect, comparing neutrophils isolated from mice expressing U2AF1-S34F to neutrophils from mice expressing U2AF1-wt. The U2AF1 mutation induced changes in splicing of genes in numerous pathways involved in RNA processing. Similar pathways are reported to be altered by MDS-associated spliceosome mutations in other cell types, suggesting that this may be one common effect of this class of mutation [32, 38, 39]. At the gene expression level, many immune signaling pathways exhibited altered gene expression induced by U2AF1 mutation, in either the absence or presence of infection. Strikingly, multiple signaling pathways involving cell migration were down-regulated in neutrophils from U2AF1-S34F mice. These pathway-level changes were driven by decreased expression of at least 18 genes. We monitored expression of the human orthologues of a handful of these genes to determine if similar changes in gene expression were induced by U2AF1 mutation in human neutrophils. Expression of two of these genes, AIF1 and PADI2, were similarly downregulated in neutrophils from MDS patients harboring U2AF1 mutations. One limitation of these studies is the small size of the human patient cohort, due to the logistics of collecting samples prospectively to isolate neutrophils. Compounding this issue is the fact that MDS is a heterogeneous disease. Thus, it is possible that some of the other candidate genes that regulate neutrophil migration might also be significantly downregulated if examined in a larger cohort.

Both AIF1 and PADI2 have been implicated in the regulation of immunity and cell migration and could contribute to the neutrophil migration defect in U2AF1-S34F mice and MDS patients. AIF1 is a calcium binding protein that affects inflammation [52]. AIF1 facilitates cell migration in multiple cell types [53–57], possibly by cross-linking actin [58, 59]. PADI2 is a peptidylarginine deiminase, an enzyme that converts arginine into citrulline. This peptide modification can have significant effects on protein function including regulators of the immune response [60, 61]. PADI2 regulates T cell migration by citrullinating various chemokines [62] and is also reported to affect various innate immune signaling pathways in myeloid cells [63–66].

While our studies demonstrate that U2AF1-S34F has profound consequences for neutrophil function, other immune cell types may also be affected by this mutation. MDS is known to affect multiple immune cell types [67], although these defects have not been characterized as thoroughly as MDS-associated neutrophil defects. For example, macrophages [68] from MDS patients are reported to have some deficiencies. It is unknown if MDS-associated U2AF1 mutations affects other myeloid cells.

It is also unknown if the immunodeficiency induced by MDS-associated U2AF1 mutations is unique to U2AF1 or if other spliceosome mutations induced similar defects. Prior studies demonstrated that there were overlaps in gene expression changes induced by these different spliceosome mutations, particularly at the pathway level [32, 38, 39]. Therefore, it is possible that similar host defense defects could be induced by these mutations, although this remains to be tested.

There has been a temporal disconnect in the studies of myeloid cell defects in MDS patients, as many of the studies focused on the function of these myeloid cells predate the identification of the panoply of mutations that drive this cancer, and thus, also predate the development of transgenic mouse models expressing these MDS-associated mutations. Thus, to our knowledge, this is the first study that directly examines how an MDS-associated mutation that drives MDS impacts myeloid cell function and host defense. In particular, our study demonstrates

that U2AF1 mutations found in MDS patients are sufficient to induce functional neutrophil defects in mice. This effect likely contributes to the increased infection risk and infection-associated morbidity in patients with MDS.

DATA AVAILABILITY

The RNAseq data generated in this study have been deposited in the Gene Expression Omnibus Database under GEO Accession number GSE209799. All other data are present in the manuscript figures, tables, and supplementary files.

CODE AVAILABILITY

Our studies use publicly or commercially available software packages for all analyses as outlined in the Supplementary Methods.

REFERENCES

1. Tefferi A, Vardiman JW. Myelodysplastic syndromes. N. Engl J Med. 2009;361:1872–85.
2. Cogle CR. Incidence and burden of the myelodysplastic syndromes. Curr Hematol Malig Rep. 2015;10:272–81.
3. Cogle CR, Craig BM, Rollison DE, List AF. Incidence of the myelodysplastic syndromes using a novel claims-based algorithm: high number of uncaptured cases by cancer registries. Blood. 2011;117:7121–5.
4. Zeidan AM, Shallis RM, Wang R, Davidoff A, Ma X. Epidemiology of myelodysplastic syndromes: why characterizing the beast is a prerequisite to taming it. Blood Rev. 2019;34:1–15.
5. Aul C, Giagounidis A, Germing U. Epidemiological features of myelodysplastic syndromes: results from regional cancer surveys and hospital-based statistics. Int J Hematol. 2001;73:405–10.
6. Ma X, Does M, Raza A, Mayne ST. Myelodysplastic syndromes: incidence and survival in the United States. Cancer. 2007;109:1536–42.
7. Dayyani F, Conley AP, Strom SS, Stevenson W, Cortes JE, Borthakur G, et al. Cause of death in patients with lower-risk myelodysplastic syndrome. Cancer. 2010;116:2174–9.
8. Madry K, Lis K, Fenaux P, Bowen D, Symeonidis A, Mittelman M, et al. Cause of death and excess mortality in patients with lower-risk myelodysplastic syndromes (MDS): a report from the European MDS registry. Br J Haematol. 2023;200:451–61.
9. Nachtkamp K, Stark R, Strupp C, Kundgen A, Giagounidis A, Aul C, et al. Causes of death in 2877 patients with myelodysplastic syndromes. Ann Hematol. 2016;95:937–44.
10. Pomeroy C, Oken MM, Rydell RE, Filice GA. Infection in the myelodysplastic syndromes. Am J Med. 1991;90:338–44.
11. Boogaerts MA, Nelissen V, Roelant C, Goossens W. Blood neutrophil function in primary myelodysplastic syndromes. Br J Haematol. 1983;55:217–27.
12. Breton-Gorius J, Houssay D, Dreyfus B. Partial myeloperoxidase deficiency in a case of preleukaemia. I. studies of fine structure and peroxidase synthesis of promyelocytes. Br J Haematol. 1975;30:273–8.
13. Martin S, Baldock SC, Ghoneim AT, Child JA. Defective neutrophil function and microbicidal mechanisms in the myelodysplastic disorders. J Clin Pathol. 1983;36:1120–8.
14. Ruutu P, Ruutu T, Repo H, Vuopio P, Timonen T, Kosunen TU, et al. Defective neutrophil migration in monosomy-7. Blood. 1981;58:739–45.
15. Ruutu P, Ruutu T, Vuopie P, Kosunen TU, de la Chapelle A. Defective chemotaxis in monosomy-7. Nature. 1977;265:146–7.
16. Ruutu P, Ruutu T, Vuopio P, Kosunen TU, de la Chapelle A. Function of neutrophils in preleukaemia. Scand J Haematol. 1977;18:317–25.
17. Nagata Y, Maciejewski JP. The functional mechanisms of mutations in myelodysplastic syndrome. Leukemia. 2019;33:2779–94.
18. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28:241–7.
19. Wahl MC, Will CL, Luhrmann R. The spliceosome: design principles of a dynamic RNP machine. Cell. 2009;136:701–18.
20. Joshi P, Halene S, Abdel-Wahab O. How do messenger RNA splicing alterations drive myelodysplasia? Blood. 2017;129:2465–70.
21. Pellagatti A, Boultwood J. Splicing factor mutations in the myelodysplastic syndromes: role of key aberrantly spliced genes in disease pathophysiology and treatment. Adv Biol Regul. 2023;87:100920.
22. Saez B, Walter MJ, Graubert TA. Splicing factor gene mutations in hematologic malignancies. Blood. 2017;129:1260–9.
23. Xu JJ, Smeets MF, Tan SY, Wall M, Purton LE, Walkley CR. Modeling human RNA spliceosome mutations in the mouse: not all mice were created equal. Exp Hematol. 2019;70:10–23.
24. Liu W, Teodorescu P, Halene S, Ghiaur G. The coming of age of preclinical models of MDS. Front Oncol. 2022;12:815037.
25. Shirai CL, Ley JN, White BS, Kim S, Tibbitts J, Shao J, et al. Mutant U2AF1 expression alters hematopoiesis and pre-mRNA splicing in vivo. Cancer Cell. 2015;27:631–43.
26. Fei DL, Zhen T, Durham B, Ferrarone J, Zhang T, Garrett L, et al. Impaired hematopoiesis and leukemia development in mice with a conditional knock-in allele of a mutant splicing factor gene U2af1. Proc Natl Acad Sci USA 2018;115:E10437–E46.
27. De Arras L, Alper S. Limiting of the innate immune response by SF3A-dependent control of MyD88 alternative mRNA splicing. PLoS Genet. 2013;9:e1003855.
28. Pollyea DA, Harris C, Rabe JL, Hedin BR, De Arras L, Katz S, et al. Myelodysplastic syndrome-associated spliceosome gene mutations enhance innate immune signaling. Haematologica. 2019;104:e388–e92.
29. Basiorka AA, McGraw KL, Eksioglu EA, Chen X, Johnson J, Zhang L, et al. The NLRP3 inflammasome functions as a driver of the myelodysplastic syndrome phenotype. Blood. 2016;128:2960–75.
30. Lee SC, North K, Kim E, Jang E, Obeng E, Lu SX, et al. Synthetic lethal and convergent biological effects of cancer-associated spliceosomal gene mutations. Cancer Cell. 2018;34:225–41 e8.
31. Smith MA, Choudhary GS, Pellagatti A, Choi K, Bolanos LC, Bhagat TD, et al. U2AF1 mutations induce oncogenic IRAK4 isoforms and activate innate immune pathways in myeloid malignancies. Nat Cell Biol. 2019;21:640–50.
32. Pollyea D, Kim H, Stevens B, Lee F, Harris C, Hedin B, et al. MDS-associated SF3B1 mutations enhance pro-inflammatory gene expression in patient blast cells. J Leuk Biol. 2021;110:197–205.
33. Choudhary GS, Pellagatti A, Agianian B, Smith MA, Bhagat TD, Gordon-Mitchell S, et al. Activation of targetable inflammatory immune signaling is seen in myelodysplastic syndromes with SF3B1 mutations. eLife. 2022;11:e78136.
34. Jiang D, Nelson ML, Gally F, Smith S, Wu Q, Minor M, et al. Airway epithelial NF-kappaB activation promotes mycoplasma pneumoniae clearance in mice. PloS One. 2012;7:e52969.
35. Dougan M, Dranoff G, Dougan SK. GM-CSF, IL-3, and IL-5 family of cytokines: regulators of inflammation. Immunity. 2019;50:796–811.
36. Martin KR, Wong HL, Witko-Sarsat V, Wicks IP. G-CSF - a double edge sword in neutrophil mediated immunity. Semin Immunol. 2021;54:101516.
37. Griffith JW, Sokol CL, Luster AD. Chemokines and chemokine receptors: positioning cells for host defense and immunity. Annu Rev Immunol. 2014;32:659–702.
38. Dolatshad H, Pellagatti A, Fernandez-Mercado M, Yip BH, Malcovati L, Attwood M, et al. Disruption of SF3B1 results in deregulated expression and splicing of key genes and pathways in myelodysplastic syndrome hematopoietic stem and progenitor cells. Leukemia. 2015;29:1092–103.
39. Pellagatti A, Armstrong RN, Steeples V, Sharma E, Repapi E, Singh S, et al. Impact of spliceosome mutations on RNA splicing in myelodysplasia: dysregulated genes/pathways and clinical associations. Blood. 2018;132:1225–40.
40. Kameritsch P, Renkawitz J. Principles of leukocyte migration strategies. Trends Cell Biol. 2020;30:818–32.
41. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013;13:159–75.
42. Nourshargh S, Alon R. Leukocyte migration into inflamed tissues. Immunity. 2014;41:694–707.
43. Davey FR, Erber WN, Gatter KC, Mason DY. Abnormal neutrophils in acute myeloid leukemia and myelodysplastic syndrome. Hum Pathol. 1988;19:454–9.
44. Moretti S, Lanza F, Spisani S, Latorraca A, Rigolin GM, Giuliani AL, et al. Neutrophils from patients with myelodysplastic syndromes: relationship between impairment of granular contents, complement receptors, functional activities and disease status. Leuk Lymphoma. 1994;13:471–7.
45. Breton-Gorius J, Houssay D, Vilde JL, Dreyfus B. Partial myeloperoxidase deficiency in a case of preleukaemia. II. defects of degranulation and abnormal bactericidal activity of blood neutrophils. Br J Haematol. 1975;30:279–88.
46. Ito Y, Kawanishi Y, Shoji N, Ohyashiki K. Decline in antibiotic enzyme activity of neutrophils is a prognostic factor for infections in patients with myelodysplastic syndrome. Clin Infect Dis. 2000;31:1292–5.
47. Brings C, Frobel J, Cadeddu P, Germing U, Haas R, Gattermann N. Impaired formation of neutrophil extracellular traps in patients with MDS. Blood Adv. 2022;6:129–37.
48. Fianchi L, Leone G, Posteraro B, Sanguinetti M, Guidi F, Valentini CG, et al. Impaired bactericidal and fungicidal activities of neutrophils in patients with myelodysplastic syndrome. Leuk Res. 2012;36:331–3.

49. Schuster M, Moeller M, Bornemann L, Bessen C, Sobczak C, Schmitz S, et al. Surveillance of myelodysplastic syndrome via migration analyses of blood neutrophils: a potential prognostic tool. J Immunol. 2018;201:3546–57.

50. Fuhler GM, Knol GJ, Drayer AL, Vellenga E. Impaired interleukin-8- and GROalpha-induced phosphorylation of extracellular signal-regulated kinase result in decreased migration of neutrophils from patients with myelodysplasia. J Leukoc Biol. 2005;77:257–66.

51. Cao M, Shikama Y, Kimura H, Noji H, Ikeda K, Ono T, et al. Mechanisms of impaired neutrophil migration by microRNAs in myelodysplastic syndromes. J Immunol. 2017;198:1887–99.

52. Deininger MH, Meyermann R, Schluesener HJ. The allograft inflammatory factor-1 family of proteins. FEBS Lett. 2002;514:115–21.

53. Autieri MV, Kelemen SE, Wendt KW. AIF-1 is an actin-polymerizing and Rac1-activating protein that promotes vascular smooth muscle cell migration. Circ Res. 2003;92:1107–14.

54. Tian Y, Kelemen SE, Autieri MV. Inhibition of AIF-1 expression by constitutive siRNA expression reduces macrophage migration, proliferation, and signal transduction initiated by atherogenic stimuli. Am J Physiol Cell Physiol. 2006;290:C1083–91.

55. Del Galdo F, Jimenez SA. T cells expressing allograft inflammatory factor 1 display increased chemotaxis and induce a profibrotic phenotype in normal fibroblasts in vitro. Arthritis Rheum. 2007;56:3478–88.

56. Liu S, Tan WY, Chen QR, Chen XP, Fu K, Zhao YY, et al. Daintain/AIF-1 promotes breast cancer proliferation via activation of the NF-kappaB/cyclin D1 pathway and facilitates tumor growth. Cancer Sci. 2008;99:952–7.

57. Jia J, Cai Y, Wang R, Fu K, Zhao YF. Overexpression of allograft inflammatory factor-1 promotes the proliferation and migration of human endothelial cells (HUV-EC-C) probably by up-regulation of basic fibroblast growth factor. Pediatr Res. 2010;67:29–34.

58. Ohsawa K, Imai Y, Kanazawa H, Sasaki Y, Kohsaka S. Involvement of Iba1 in membrane ruffling and phagocytosis of macrophages/microglia. J Cell Sci. 2000;113:3073–84.

59. Sasaki Y, Ohsawa K, Kanazawa H, Kohsaka S, Imai Y. Iba1 is an actin-cross-linking protein in macrophages/microglia. Biochem Biophys Res Commun. 2001;286:292–7.

60. Curran AM, Naik P, Giles JT, Darrah E. PAD enzymes in rheumatoid arthritis: pathogenic effectors and autoimmune targets. Nat Rev Rheumatol. 2020;16:301–15.

61. Wu Z, Li P, Tian Y, Ouyang W, Ho JW, Alam HB, et al. Peptidylarginine deiminase 2 in host immunity: current insights and perspectives. Front Immunol. 2021;12:761946.

62. Loos T, Mortier A, Gouwy M, Ronsse I, Put W, Lenaerts JP, et al. Citrullination of CXCL10 and CXCL11 by peptidylarginine deiminase: a naturally occurring post-translational modification of chemokines and new dimension of immunoregulation. Blood. 2008;112:2648–56.

63. Lee HJ, Joo M, Abdolrasulnia R, Young DG, Choi I, Ware LB, et al. Peptidylarginine deiminase 2 suppresses inhibitory kappaB kinase activity in lipopolysaccharide-stimulated RAW 264.7 macrophages. J Biol Chem. 2010;285:39655–62.

64. Wu Z, Deng Q, Pan B, Alam HB, Tian Y, Bhatti UF, et al. Inhibition of PAD2 improves survival in a mouse model of lethal lps-induced endotoxic shock. Inflammation. 2020;43:1436–45.

65. Yu HC, Tung CH, Huang KY, Huang HB, Lu MC. The essential role of peptidylarginine deiminases 2 for cytokines secretion, apoptosis, and cell adhesion in macrophage. Int J Mol Sci. 2020;21:5720.

66. Wu Z, Tian Y, Alam HB, Li P, Duan X, Williams AM, et al. Peptidylarginine deiminases 2 mediates caspase-1-associated lethality in pseudomonas aeruginosa pneumonia-induced sepsis. J Infect Dis. 2021;223:1093–102.

67. Fozza C, Crobu V, Isoni MA, Dore F. The immune landscape of myelodysplastic syndromes. Crit Rev Oncol Hematol. 2016;107:90–9.

68. Han Y, Wang H, Shao Z. Monocyte-derived macrophages are impaired in myelodysplastic syndrome. J Immunol Res. 2016;2016:5479013.

AUTHOR CONTRIBUTIONS

NJG, KCM, CH, JRK, BPO, JM, FFYL, CP, JO, and EAW performed experiments and analyzed data. CMM and DAP provided key samples. NJG, KCM, WJJ, EMP, DAP, and SA conceptualized the project, supervised results, and wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

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ADDITIONAL INFORMATION

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