Transcriptional Regulation of Emergency Granulopoiesis in Leukemia

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Neutropenic conditions are prevalent in leukemia patients and are often associated with increased susceptibility to infections. In fact, emergency granulopoiesis (EG), a process regulating neutrophil homeostasis in inflammatory conditions and infections, may occur improperly in leukemic conditions, leading to reduced neutrophil counts. Unfortunately, the mechanisms central to dysfunctional EG remain understudied in both leukemia patients and leukemic mouse models. However, despite no direct studies on EG response in leukemia are reported, recently certain transcription factors (TFs) have been found to function at the crossroads of leukemia and EG. In this review, we present an update on TFs that can potentially govern the fate of EG in leukemia. Transcriptional control of Fanconi DNA repair pathway genes is also highlighted, as well as the newly discovered role of Fanconi proteins in innate immune response and EG. Identifying the TFs regulating EG in leukemia and dissecting their underlying mechanisms may facilitate the discovery of therapeutic drugs for the treatment of neutropenia.

Keywords: emergency granulopoiesis, Fanconi DNA repair pathway, infections, leukemia, neutropenia, neutrophils

INTRODUCTION

Bacterial and fungal infections are a leading cause of morbidity and mortality in acute leukemia patients (1, 2). A recent study reported that 71.9% of chronic lymphocytic leukemia (CLL) patients developed infections with a mortality rate of 37.5% (3). Abnormal proliferation of myeloid cells occurs and immature leukocytes accumulate in bone marrow in a cancer microenvironment that can also inhibit antigen-specific T cell response (4). Therefore, leukemia patients are particularly at a high risk for infectious complications. Highly intensive chemotherapy results in prolonged neutropenia, rendering the patients extremely susceptible to microbial infections (5). Prolonged periods of neutropenia proportionately increase the risk of severe infections, which can be exacerbated with the relapse of disease (6, 7).

Neutrophils are key mediators of the early inflammatory response at the time of an infection, and reduced neutrophil counts can lead to life-threatening infections (8). Neutrophil homeostasis is differentially regulated during steady state and infectious episodes. During infections or inflammation, circulating neutrophils become significantly elevated in a process called emergency granulopoiesis.
(EG). This process involves enhanced generation of neutrophils in the bone marrow through increased myeloid progenitor cell proliferation (9). Neutrophil mobilization is also increased in response to elevation in circulatory granulocyte colony stimulating factor (G-CSF) levels resulting in effective clearance of bacterial and fungal pathogens (10).

Execution of EG occurs in four different stages: (i) it commences with an increase in the peripheral neutrophil count due to vascular demargination and releases from the bone marrow mediated by disruption of CXCL12/CXCR4 signaling (11–15). This phase is accompanied by (ii) de novo generation of neutrophils from increased myeloid progenitor cell proliferation (9) and (iii) their accelerated differentiation by S-phase shortening of the cell cycle stabilized by the Fanconi pathway (16) followed by (iv) termination of EG response, which is partly mediated by interferon regulatory factor (IRF8) (17). A recent study demonstrated that, in cancer chemotherapy-induced neutropenia (CCIN), the neutrophils generated during EG response were functionally immature in both humans and mouse models and displayed weak bactericidal activity in both (18). Studies evaluating the defects in completion of the EG response during leukemic conditions are scarce; however, certain transcription factors (TFs) have been identified, which share an overlapping role in leukemia and innate immunity. The purpose of this review is to characterize the role of these TFs in leukemia and their link to the EG response. Recently discovered role of the Fanconi DNA repair pathway in innate immunity will also be discussed.

**REGULATION OF EMERGENCY GRANULOPOIESIS**

Emergency granulopoiesis is regulated by various endogenous and exogenous factors. Our knowledge of endogenous factors, predominantly transcriptional regulators, has increased significantly over the past decade. Various TFs play a pivotal role in modulating both EG and leukemia development. Recent studies have shown that dysregulation of these TFs leads to perturbed granulopoiesis along with an aggravation of leukemic state. The role of TFs in normal hematopoiesis, EG, and leukemogenesis is discussed in the following section. Table 1 presents a list of the TFs discussed here.

**HOXA10-ROLE IN IMMUNE CELL DEVELOPMENT AND LEUKEMIA**

HOXA10 is a homeodomain-containing TF which is a part of the A cluster on chromosome 7. HOXA10 is considered a master regulator of postnatal hematopoietic development that controls hematopoietic stem cell (HSC) self-renewal, the development of lymphoid and erythroid/megakaryocyte cells, as well as platelet biogenesis (19–21). It is also abundantly expressed in myeloid progenitors, where it influences myelopoiesis (22–24) and in phagocytic cells, where it represses transcription of the genes encoding p67phox (NADPH oxidase subunit) and gp91phox (cytochrome b subunit beta), thereby influencing its effector functions (25). However, overexpression of HOXA10 in murine bone marrow has been shown to induce a myeloproliferative disorder (MPD) involving expansion of the committed myeloid progenitors, which later evolves into acute myeloid leukemia (AML) (26). Leukemias with chromosomal translocations of the mixed lineage leukemia 1 (MLL1) gene are characterized by increased and sustained transcription of a group of HOX genes (including HOXA10), as fusion proteins generated by MLL1 gene translocations lack ubiquitination/degradation domains (27). Mice transplanted with bone marrow expressing an MLL-fusion protein or overexpressing HOXA10 develop AML (26, 28–30). In a recent study, the MLL-ELL fusion protein was found to increase expression of HOXA9 and HOXA10 directly, by interaction with their promoters, and indirectly via fibroblast growth factor 2 (FGF2), β-catenin, and caudal-type homebox 4 (CDX4) (31).

**HOXA10-ROLE IN EMERGENCY GRANULOPOIESIS THROUGH HOXA10-TRIAD1 INTERACTION**

Apart from the leukemogenic role of HOXA10, its role in regulating EG has also been elucidated recently. HOXA10−/− mice showed a fatal EG response, which was rescued by re-expression of TRIAD1 (alias ARIH2) (32). TRIAD1, encoded by the gene ARIH2 (32), is a ubiquitin ligase that regulates myelopoiesis by inhibiting proliferation of myeloid cells (32, 33). In one study, hematopoietic deficiency of ARIH2 caused lethal activation of the immune system. Sustained activity of NF-κB (RELA) was found in ARIH2−/− mice showing a protective role in EG (45). This phase is accompanied by (ii) de novo generation of neutrophils from increased myeloid progenitor cell proliferation (9) and (iii) their accelerated differentiation by S-phase shortening of the cell cycle stabilized by the Fanconi pathway (16) followed by (iv) termination of EG response, which is partly mediated by interferon regulatory factor (IRF8) (17). Important during G-CSF signaling (68) Constitutively active STAT3 in AML cell lines (71)

**TABLE 1** | Transcription factors with intersecting roles in EG and leukemia.

| Transcription Factors | Functions |
|-----------------------|-----------|
| HOXA10                | • Postnatal hematopoietic development and HSC self-renewal (19)  
• Development of lymphoid/erythroid/megakaryocyte cells (21)  
• AML development (25)  
• Termination of EG response by activating TRIAD1 (32) |
| CEBPβ                 | • Granulocyte proliferation and differentiation during EG (65)  
• Promotion of leukemogenesis by LIP isoform (44) |
| IRF8                  | • Expression of proinflammatory cytokines (54)  
• Macrophage differentiation (57)  
• Termination of EG response (17)  
• Tumor suppression (50) |
| STAT3                 | • Important during G-CSF signaling (68)  
• Constitutively active STAT3 in AML cell lines (71) |
| STAT5                 | • Anti-apoptotic role during myeloid differentiation (69)  
• Oncogenesis (99) |

AML, acute myeloid leukemia; EG, emergency granulopoiesis; G-CSF, granulocyte colony stimulating factor; HSC, hematopoietic stem cell.
up-regulation of TRIAD1 by HOXA10 and presents protein ubiquitination/degradation as a novel mechanism of regulating EG response by HOXA10. Increased TRIAD1 expression degrades FGFR1, thereby reducing the levels of FGF2 and terminating the effect of FGF2 on myeloid progenitor expansion and phagocyte effector function. All these processes culminate in termination of EG, with HOXA10 being the prime mediator. Moreover, in the bone marrow of HOXA10−/− mice, TRIAD1 expression was only slightly decreased at steady state but TRIAD1 expression was totally absent during EG, suggesting the specific role of HOXA10 during EG. Transcription of ARIH2 and expression of TRIAD1 during EG was regulated by tyrosine phosphorylation of HOXA10. Thus, this study elucidated the induction of protein degradation via TRIAD1 as a novel immune modulatory mechanism of HOXA10 (32).

Based on these studies, it can be speculated that, during EG in leukemia, overexpression of HOXA10 leads to sustained activation of TRIAD1, which favors a suppressed EG state, thus identifying one factor that may cause neutropenia in leukemia patients and make them more susceptible to infections (Figure 1). On the other hand, higher leukemia transformations have been reported in severe congenital neutropenia (SCN) patients who required higher G-CSF doses (35), indicating that there are common TFs that mediate leukemogenesis and granulopoiesis.

CCAAT/ENHANCER BINDING PROTEIN BETA—ROLE IN IMMUNE CELL DEVELOPMENT AND LEUKEMIA

CCAAT/Enhancer Binding Protein beta (C/EBP-β) is a basic leucine zipper (bZIP) domain-containing TF that plays an important role in regulating immune and inflammatory responses (36–39). Both leukemia suppressor and pro-oncogenic roles of C/EBP-β have been reported. C/EBP-β was shown to suppress the leukemogenic potential of 32D-BCR-ABL cells by inducing granulocytic differentiation and by inhibiting cell proliferation. Low C/EBP-β expression is observed in the blast crisis stage of chronic myelogenous leukemia (CML) and is inversely correlated with BCR-ABL tyrosine kinase levels, suggesting that there may be therapeutic potential in restoring its activity in CML-BC (40).

In acute promyelocytic leukemia (APL), treatment with all-trans retinoic acid (ATRA) reverses promyelocytic leukemia-retinoic acid receptor α (PML-RARα)-mediated differentiation block at the promyelocyte stage resulting in mature neutrophil-like cells (41, 42). C/EBP-β is upregulated in the presence of PML-RARα during ATRA treatment and promotes the proliferation and differentiation of APL cells, thereby showing a potential anti-cancer role (42).

C/EBP-β exists as several isoforms due to alternative translation initiation: full-length C/EBP-β liver activating protein* (LAP*), a slightly shorter isoform of LAP that lacks the first 21 amino acids and a short isoform of liver inhibitory protein (LIP). LAP* and LAP are trans-activators, whereas LIP is a transcriptional repressor. The relative abundance of LIP and LAP C/EBP-β isoforms mediated through the regulation of translation initiation is important in determining cell fate by controlling proliferation and differentiation (43). In contrast to the leukemia suppressive effect of C/EBP-β, its LIP isoform was shown to promote leukemogenesis in a mouse bone marrow transplantation system by collaborating with Ecotropic viral integration site 1 (Evi1) which is one of the master regulators of AML development. However, experiments performed on human whole BM cells from AML
patients revealed that Evi1 closely correlated with both LAP* and LIP expression (44).

**CCAAT/ENHANCER BINDING PROTEIN BETA—ROLE IN EMERGENCY GRANULOPOIESIS**

Hirai et al. showed that C/EBP-α is required for steady-state granulopoiesis whereas C/EBP-β is essential for EG (45–47). Only C/EBP-β expression (and not that of other C/EBPs) was upregulated in GMPs after cytokine treatment (45). Using C/EBP β/−/− bone marrow cells, it was found that C/EBP-β is involved in cytokine (G-CSF, GM-CSF, IL-3, and IL-6)-induced myeloid proliferation, suggesting that C/EBP-β is required to couple proliferation and differentiation of granulocytes under stress or emergency situations, thereby producing more mature granulocytes (45). The specific role of LAP*, LIP, and LAP isoforms of C/EBP-β in EG is not clear; however, exploring this may help target the specific isoform for both anti-leukemic and anti-neutrophenic effects.

**IRF8 (ALSO KNOWN AS INTERFERON CONSENSUS SEQUENCE BINDING PROTEIN)—ROLE IN IMMUNE CELL DEVELOPMENT AND LEUKEMIA**

The critical role of IRF8 in innate immune response and oncogenesis has been described extensively (48–51). In response to pattern-recognition receptors (PRR) activation, IRF8 induces the expression of proinflammatory cytokines through TLR9-MyD88-dependent signaling (52–55). IRF8 inhibits cell growth and promotes apoptosis in myeloid cells and drives their differentiation toward macrophages while inhibiting neutrophil production (56, 57). IRF8 plays an important role in myeloid cell development, as has been demonstrated by a systemic expansion of neutrophils followed by a fatal blast crisis, resembling human CML in IRF8−/− mice (50). These mice also exhibit increased progenitor cell numbers with enhanced responsiveness to granulocyte-macrophage colony stimulating factor (GM-CSF) and G-CSF. In contrast, responsiveness to macrophage colony stimulating factor (M-CSF) was reduced in IRF8−/− progenitors, implying a role for IRF8 in driving toward the macrophage lineage (57, 58). A tumor suppressor role has been described for IRF8, as very low or absent IRF8 mRNA was found in the peripheral blood of the majority of human myeloid leukemias. Sorted B-cells derived from CML patients also showed the absence of IRF8 mRNA (59). Moreover, high IRF8 mRNA levels were found only in those CML patients who were classified as “good” cytogenetic responders to interferon-α therapy and not in the poor responders (60). One of the target genes of IRF8 is Fas-associated phosphatase 1 (FAP1; the PTPN13 gene), which shares a reciprocal expression profile with IRF8 at all clinical stages of CML. Impaired IRF8 expression in BCR−ABL + myeloid progenitor cells contributed to FAP1-dependent Fas resistance. As Fas resistance contributes to persistence and expansion of CML leukemic stem cells (LSCs), it led to imatinib resistance in BCR − ABL + GMPs through the reduction of IRF8 expression and increased FAP1 expression (61).

IRF8 also represses the Growth Arrest Specific 2 gene (GAS2), which encodes a calpain inhibitor that is involved in cell proliferation and survival. GAS2 expression in BCR-ABL+ cells stabilizes β-catenin, which is a calpain substrate (62). In addition to β-catenin, calpain has other substrates that are involved in the pathogenesis of CML including signal transducer and activator of transcription 5 (STAT5) (63). In a recent study, BCR-ABL-induced SHP2-dependent dephosphorylation of IRF8 was found to impair repression of GAS2, leading to decreased calpain activity and thereby an increase in its substrate protein STAT5, which in turn represses IRF8 promoter. This novel feedback mechanism involving calpain enhances leukemogenesis by increasing STAT5 and repressing IRF8. Hence, therapeutic upregulation of IRF8 can reduce persistent LSCs during treatment of CML with BCR-ABL-targeted tyrosine kinase inhibitors (TKIs) (64). In another study, induction of IRF8 and repression of β-catenin was found upon arachidonate 15-lipoxygenase (15-LO) inhibition by PD146176 in K562 cells, implicating another mechanism where IRF8 may be involved in eradicating CML LSCs (65). In addition to the role of IRF8 in myeloid leukemia, recently its role in suppressing acute lymphoblastic leukemia has been described. Mice deficient for both PU.1 and IRF8 developed pre-B-ALL at high frequency by reducing the expression of known tumor suppressors, including SPI-B, IKAROS, and BLNK (66).

**IRF8 AS A REGULATORY COMPONENT OF EMERGENCY GRANULOPOIESIS**

In addition to its leukemia suppressor role, IRF8 plays an important regulatory role in innate immune response (51). Some of the target genes of IRF8 include FANCC and FANCF, encoding Fanconi C and F, respectively, which contribute to the Fanconii DNA repair pathway activation during infectious challenge (16, 67). IRF8 activates FANCF cis element in differentiating myeloid cells, thereby protecting them from genotoxic stress associated with differentiation (67). IRF8 was also found to bind and activate a cis element in the proximal FANCC promoter. Re-expression of FANCC rescued DNA repair in IRF8-deficient myeloid cells. Furthermore, IRF8 activates FANCC in murine myeloid progenitor cells upon stimulation with IL-1β and G-CSF, cytokines that are essential for EG (16). As rapid expansion due to S-phase shortening of granulocyte/monocyte progenitor (GMP) populations occurs during the cell cycle, IRF8 contributes to genomic stability during EG through the Fanconi pathway (16, 67) (Figure 2).

In addition to this, a more specific role of IRF8 in the termination of EG response was recently described. In IRF8−/− mice, sustained granulocyte production was found in response to EG via increased expression of FAP1 and GAS2 in bone marrow myeloid progenitor cells, which leads to decreased FAS sensitivity and increased β-catenin activity in these cells. This implies that IRF8 mediates termination of the EG response by increasing FAS-induced apoptosis and decreasing β-catenin activity in these cells, thereby limiting granulocyte proliferation (17). However, repeated episodes of EG did not increase granulocytes in IRF8−/− mice; instead, an accumulation of myeloid blasts was found leading to AML development (Figure 3). This effect is mediated
IRF8 plays an important role in myeloid cell development during emergency granulopoiesis (EG) via regulating Fanconi DNA repair proteins. Some of the target genes of IRF8 include FANCC and FANCF, encoding Fanconi C and Fanconi F, respectively. These Fanconi proteins contribute to Fanconi DNA repair pathway activation during EG and protect cells from the genotoxic stress of myelopoiesis during rapid proliferation phase.

Repeated episodes of emergency granulopoiesis (EG) lead to leukemia development in IRF8−/− (ICSBP−/−) mice. A sustained expansion of neutrophils was observed in IRF8−/− mice in response to EG through increased expression of FAP1 and GAS2 in bone marrow myeloid progenitors. However, repeated episodes of EG did not increase granulocytes in IRF8−/− mice, but accumulation of myeloid blasts was found, leading to acute myeloid leukemia (AML) development. Reduced expression of FANCC and FANCF, which are IRF8 target proteins, contribute by increasing sensitivity to DNA damage in bone marrow myeloid progenitors.

From these observations, it can be inferred that there may be a dual impact of reduced IRF8 on neutrophil production in leukemic conditions during an EG response. Reduced IRF8 would prolong the termination step of EG (by increasing FAP1 and GAS2 expression), thereby causing granulocytosis with functionally incompetent cells (17). Simultaneously, it will also promote apoptosis of myeloid progenitors during the rapid proliferation step via decreased expression of FANCC and F that rescue collapsed and stalled replication forks during DNA replication. This leads to reduced neutrophil count or enhanced leukemogenesis due to accumulation of mutations. Therefore, gene regulation of Fanconi DNA repair proteins by IRF8 presents an important link between innate immune response and the leukemia suppressor role of IRF8.

STAT PROTEINS

STAT3—Role in Immune Cell Development and Leukemia

STAT3 is one of the most important TFs phosphorylated by Janus kinases after G-CSF-induced dimerization of G-CSFR (68, 69). G-CSF and IL-6 are potent activators of STAT3 in hematopoietic progenitor cells and neutrophils (69, 70). Constitutively active STAT3 has been reported in human AML cell lines and in pretreatment bone marrow samples of AML patients, where it was associated with shorter disease-free survival (71, 72). Inhibiting
the G-CSF-induced STAT3 phosphorylation by a small-molecule STAT3 inhibitor C188-9 induced apoptosis in AML cell lines and primary pediatric AML samples (73). Another STAT3 inhibitor, BP-5-087, in combination with imatinib, reduced the survival of primary tyrosine kinase inhibitor (TKI)-resistant stem and progenitor cells, as activation of pSTAT3 (at amino acid position Y705) is important for BCR-ABL1 kinase-independent TKI resistance (74).

STAT3 interacts directly with c-Jun, as has been reported using the yeast two-hybrid system (75). STAT3, in cooperation with c-JUN and c-FOS, activates the IL-6 response element (IRE) (76). Elevated c-JUN expression has been linked to AML, where it inactivates C/EBP-α via leucine zipper domain interaction. This interaction and C/EBP-α inactivation are necessary to induce proliferation in AML (77). The role of c-JUN during EG has not been explored yet; however, its interaction with STAT3 and IRE activation point toward its involvement as a positive regulator of EG. c-JUN may act as an additional TF that lies at the intersection of EG and leukemia and may enhance the development of leukemia along with skewing of the EG response.

STAT3—Role in Emergency Granulopoiesis

In response to G-CSF, STAT3 accelerates granulocyte cell-cycle progression (G1–S phase transition) and terminal maturation by regulating C/EBP-β, which is an important TF in the EG response. STAT3 and C/EBP-β co-regulate c-MYC through direct interaction with its promoter and displacement of C/EBP-α during demand-driven granulopoiesis (78). A recent study has shown the involvement of STAT3 and C/EBP-β in the activation of FANCC transcription which contributes to DNA repair during EG (79).

STAT3 REGULATES SUPPRESSOR OF CYTOKINE SIGNALING-3 (SOCS3) EXPRESSION

Studies done in STAT3-deficient mice show neutrophilia and hyper-responsiveness of myeloid cells to G-CSF. This was related to reduced SOCS3 expression, thereby showing the role of STAT3 in inducing SOCS3, which in turn acts as a negative regulator of proliferative signals from G-CSF signaling required during EG (80). SOCS3 deficiency significantly increases STAT3 activation in response to in vivo administration of G-CSF which leads to toxic effects (81). Similarly, in another study on SOCS3-deficient hematopoietic progenitor cells, STAT3 and C/EBP-β activation was increased in response to G-CSF and IL-6 (82). As mentioned above, STAT3 induces C/EBP family TFs and interacts with them to augment the effect of G-CSF signaling (83). SOCS3 is recognized as a negative regulator of G-CSF signaling and EG in myeloid cells (81, 84). Constitutive STAT3 phosphorylation was shown to constitutively activate SOCS3 expression in AML blasts isolated from patients (85). An inefficient EG response during infectious episodes can be expected from constitutively increased expression of SOCS3 under such leukemic conditions. Another study on STAT3-deficient mice showed a failure to accumulate immature granulocytes in the bone marrow after G-CSF exposure, thereby leading to an increase in the ratio of mature to immature neutrophils. However, immature granulocytes are needed during EG for increased neutrophil mobilization. The study characterized impairment in acute neutrophil mobilization which is independent of SOCS3, indicating diverse signaling pathways in response to G-CSF (86).

Recently, activating somatic STAT3 mutations located in the Src homology 2 (SH2) domain have been described in T-cell large granular lymphocytic leukemia with a high frequency of 40% (31 of 77 patients). These patients presented more often with neutropenia than patients without these mutations (87). As discussed earlier, this could be due to the induction of SOCS3 by activated STAT3, which negatively regulates G-CSF signaling (81). Based on these observations, constitutive activation of STAT3 might contribute to failure of the EG response in leukemic patients resulting in neutropenia and increased susceptibility to infections.

Targeting STAT3 can provide highly specific approach to treat failed EG responses in leukemia. Evidently, there is a growing list of STAT3 inhibitors under clinical evaluation which rely on direct or indirect targeting mechanisms (88). Most common STAT3 targeting approaches include inhibition of tyrosine kinases that phosphorylate and upregulate STAT3 activity (89). Others include STAT3 SH2 domain (dimerization) inhibitors thereby preventing the formation of functional STAT3 dimers; STAT3 DNA binding domain inhibitors that prevent binding of STAT3 to its DNA promoter, STAT3 gene expression oligonucleotide inhibitors which compete for STAT DNA binding (88), and STAT3 N-terminal domain inhibitors that disrupt protein–protein interactions between STAT3 and other TFs (90). Therefore, in addition to the possible therapeutic potential of STAT3 inhibitors in cancer treatment they can also be considered for their efficacy in treating infectious episodes in leukemia.

STAT5—ROLE IN IMMUNE CELL DEVELOPMENT AND LEUKEMIA

STAT5 is an important STAT family protein that is activated by G-CSF (91). STAT5a and STAT5b are two forms of STAT5 that are encoded by two distinct but closely related genes (92) and are activated by tyrosine phosphorylation through many factors and cytokines like prolactin, growth hormone (93), erythropoietin (94), thrombopoietin (95), interleukin 3 (IL-3), GM-CSF (96), and interleukin 2 (IL-2) (97). An anti-apoptotic role of STAT5 has been documented during differentiation of myeloid progenitors. In a study by Kieslinger et al. (98), primary chicken myeloblasts expressing dominant-negative alleles of STAT5 were unable to generate mature cells due to increased apoptosis during differentiation. Bone marrow cells from STAT5a/STAT5b-deficient mice showed increased apoptosis during GM-CSF-dependent maturation in vitro. This apoptotic cell death was rescued by ectopic expression of BCL-X, thereby showing an important role of STAT5 during cytokine-dependent differentiation of myeloid progenitors during inflammation (98).

STAT5 signaling can promote oncogenesis (99) and hyper-activated STAT5 has been implicated in various leukemia types
such as BCR-ABL-induced CML and AML, and in MPDs, such as chronic myelomonocytic leukemia and polycythemia vera (99–101).

Constitutive STAT5 activation has been demonstrated to be essential in a mouse model of MPD induced by TEL-JAK2 fusion protein. TEL-JAK2 fusion protein-mediated constitutive STAT5 activation is essential in a mouse model of MPD (102). Constitutively active STAT5 mutant in CD34−c-Kit+Sca-1+ lineage marker− (CD34 KSL) HSCs induced fatal MPD in a mouse model, implying the crucial role of STAT5 in self-renewal of HSCs during MPD development (99). These studies show that STAT5 is involved in both neutrophic conditions and development of hematologic malignancies. Therapeutic approaches to target caSTAT5 are being studied, like the small molecule bromodomain inhibitor JQ1, which reduces STAT5 function in leukemia and lymphoma cells with caSTAT5 (103).

STAT5-POSSIBLE REGULATORY ROLE IN EMERGENCY GRANULOPOIESIS

STAT5-null (STAT5A and STAT5B) HSCs in mice show an impaired repopulation potential and disrupt multilineage hematopoiesis in the bone marrow, including a reduction in neutrophil progenitors and mature neutrophils (104). However, constitutively active STAT5 (caSTAT5a) and not wild-type STAT5a is associated with inhibition of lymphoid enhancer-binding factor 1 (LEF-1) in CD34+ cells of congenital neutropenia (CN) patients. LEF-1 positively regulates G-CSF-triggered granulopoiesis by promoting proliferation and differentiation of granulocyte precursors (105). caSTAT5a inhibits LEF-1-dependent autoregulation of LEF-1 gene promoter by binding to the LEF-1 protein, recruiting Nemo-like kinase and the E3 ubiquitin-ligase NARF to LEF-1. This leads to LEF-1 ubiquitination and a reduction in LEF-1 protein levels, severely affecting neutrophil production. Interestingly, sustained elevation of phospho-STAT5 in CD34+ cells was observed in the CN patients compared to healthy controls, which was correlated to the development of AML (100).

EMERGING ROLE OF FANCONI ANEMIA (FA) DNA REPAIR PATHWAY INTERCONNECTS LEUKEMIA AND INNATE IMMUNE RESPONSE

To date, 19 genes belonging to FA complementation groups are known (A, B, C, D1, D2, E, F, G, I, J, L, M, N, O, P, Q, R, S, T) (106, 107). The FA pathway is required to repair DNA interstrand crosslinks (ICLs) which involves nucleotide excision repair (NER), translesion synthesis (TLS), and homologous recombination (HR) (108). ICLs affect DNA replication and transcription through stalling of replication forks and preventing strand separation. Therefore, un repaired ICLs lead to DNA breakage and chromosomal rearrangements resulting in cellular apoptosis or accumulation of mutations (109). In this regard, FA pathway plays an important role in genome maintenance by repairing DNA damage during replication stress responses, especially during S phase of the cell cycle (110). Hypersensitivity to DNA damage agents that induce ICL is observed in cells deficient in any component of FA pathway (111, 112). Cells undergo G2/M arrest and chromosomal breakage on treatment with mitomycin C (MMC) or diepoxybutane (DEB) (113, 114). In humans, germ-line inactivation of any FA gene predisposes them to increased sensitivity to ICLs, thereby resulting in bone marrow failure (BMF) and cancer development (115–117).

The FA pathway is also important to maintain hematopoietic stem and progenitor cells (HSPC) population. In FA patients, p53/p21 activation and G0/G1 cell cycle arrest occurs in HSPC leading to BMF in FA, whereas p53 deficiency rescued the HSPC defects in human FA cells. Therefore, HSPC instability in FA patients increases their chances to develop AML (118). K-RAS or c-MYC induced oncogenic stress caused a short-lived response in mice deficient for the FA core complex components FANCA or FANCC. Downregulation of Protein Arginine Methyltransferase 5 (PRMT5) led to compromised K-ras1207-induced arginine methylation of p53 in FANCA deficient cells, thereby demonstrating an arginine methylation-dependent FA-p53 interaction, as forced expression of PRMT5 in FANCA−/− HSPCs prolonged oncogenic response and delayed leukemia development in irradiated recipient mice (119). In another recent study, FANCC deficient aging mice developed hematologic malignancies that precede genomic instability and hematopoietic chromosomal instability or aneuploidy (120). This is further supported by the observation that AML displays an acquired decrease in expression of Fanconi proteins. Role of FANCF in leukemia suppression has also been shown. CHRF-288 (an AML cell line) exhibits a cellular FA phenotype due to lack of FANCF expression, which is corrected by a FANCF-expressing plasmid. FANCF is localized in a hot-spot region for somatic hypermethylation (11p15); therefore, gene silencing due to hypermethylation of the promoter region of the FANCF gene explains the absence of FANCF protein in CHRF-288 cell line (121). FANCF is also an IRF8 target gene that provides genomic stability to myeloid cells from DNA cross-link damage during proliferation and differentiation stages (67). Mitomycin C-induced DNA damage was increased in IRF8 deficient primary murine bone marrow cells, which was rescued by FANCF overexpression (17). Together, these findings strongly suggest a functional cross-talk between cell proliferation and DNA repair pathways.

Until now, the major roles of Fanconi pathway have been shown in maintenance and proliferation of HSPC (122); tumor suppression (123–127); stabilizing the replication fork during S phase; and DNA repair processes to protect against unwanted mutations (108, 128–130). However, its role in innate immune response has emerged recently. As mentioned previously, FANCC is an IRF target gene and IRF8+/− mice succumbed to infectious challenge with failed leukocytosis response (16, 50, 131). This observation implies that EG will be impaired in response to infectious or inflammatory challenge. As Fanconi proteins protect cells from genotoxic stress of myeloepoiesis during rapid proliferation phase, their potential role in EG response is plausible. FANCC-deficient mice showed an abnormal response to EG and developed progressive neutropenia and anemia which resulted from excess apoptosis of bone marrow HSCs and myeloid progenitors.
These effects led to failed EG episodes which contributed to BMF and suggest that FANCC expression and Fanconi pathway is enhanced during infectious episodes and is an essential element of EG response. Upon treatment with an essential EG cytokine IL-1β, FANCC, FANCI, and RAD51 were enriched in the chromatin fraction of murine myeloid progenitor cells, signifying their increased expression and diverse DNA repair processes initiated during EG. Moreover, treatment with an IL-1R antagonist (anakinra) in alum-treated FANCC-knockout mice ameliorated BMF (16).

These observations again point toward the overlapping role of FANCC protein in leukemia suppression and completing a successful EG response. Lack of this protein resulted in enhanced susceptibility to AML development and failed EG in response to infectious challenge thereby leading to neutropenia (16).

CONCLUSION

From the studies reviewed here, it can be inferred that the alterations in the expression of TFs which promote leukemia also cause an improper EG execution leading to neutropenia.

Neutropenic conditions will result in increased susceptibility to infections. That is why severe sepsis is observed in cancer patients at a much higher rate than in non-cancer patients (132). So, a counter question can also arise: do neutropenic conditions lead to leukemia? An example can be found in myelodysplastic syndromes where the neutropenic patients are at a higher risk for leukemia. The findings, albeit very few, point to a role of TFs in the pathogenesis of these diseases and it will be of interest to study if failed EG episodes predisposes a person toward leukemia. Identifying TFs that can revert the disease phenotype or selectively treat neutropenia in conjunction with drugs used for leukemia is another highly promising strategy that may be tested. Therefore, targeting intersecting TFs can be of therapeutic value to treat lymphoid and myeloid leukemia and associated disorders including neutropenia.

AUTHOR CONTRIBUTIONS

SH designed and drafted the manuscript. SH and ARN wrote the manuscript. AR and SH finalized the figures and table. All authors read and approved the final manuscript.

REFERENCES

1. Alexander S, Nieder M, Zerr DM, Fisher BT, Dvorak CC, Sung L. Prevention of bacterial infection in pediatric oncology: what do we know, what can we learn? *Pediatr Blood Cancer* (2012) 59(1):16–20. doi:10.1002/pbc.23416
2. Bhatt VR, Viola GM, Ferrajoli A. Invasive fungal infections in acute leukemia. *Ther Adv Hematol* (2011) 2(4):231–47. doi:10.1177/2040620011410098
3. Williams AM, Baran AM, Meacham PJ, Feldman MM, Valencia HE, Newsom-Stewart C, et al. Analysis of the risk of infection in patients with chronic lymphocytic leukemia in the era of novel therapies. *Leuk Lymphoma* (2017) 59(3):625–32. doi:10.1080/10428194.2017.1347931
4. Almand B, Clark JJ, Nikitina E, van Beynen J, English NR, Knight SC, et al. Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. *J Immunol* (2001) 166(1):678–89. doi:10.4049/jimmunol.166.1.678
5. Young LS. Management of infections in leukemia and lymphoma. In: Rubin RH, Young LS, Van Furrh R, editors. *Clinical Approach to Infection in the Compromised Host*. Boston, MA: Springer US (2002). p. 497–526.
6. Bodey GP. The changing face of febrile neutropenia—from monotherapy to mOULDs to mucoctosis. Fever and neutropenia: the early years. *J Antimicrob Chemother* (2009) 63(Suppl 1):i3–13. doi:10.1093/jac/dkp074
7. Rekha C, Morgan H, Stephen S. Sepsis - an ongoing and significant challenge. In: Azvedo L, editor. *Infections in Leukemia*. Bijjeka: InTech (2012). 420 p.
8. Wirths S, Bugl S, Kopp HG. Neutrophil homeostasis and its regulation by danger signaling. *Blood* (2014) 123(23):3563–6. doi:10.1182/blood-2013-11-516260
9. Manz MG, Boettcher S. Emergency granulopoiesis. *Nat Rev Immunol* (2014) 14(5):302–10. doi:10.1038/nri3660
10. Lieschke GJ, Grail D, Hodgson G, Metcalf D, Stanley E, Cheers C, et al. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. *Blood* (1994) 84(6):1737–46.
11. Semerad CL, Liu F, Gregory AD, Stumpf K, Link DC. G-CSF is an essential regulator of neutrophil trafficking from the bone marrow to the blood. *Immunity* (2002) 17(4):413–23. doi:10.1016/S1074-7613(02)00424-7
12. Semerad CL, Christopher MJ, Liu F, Short B, Simmons PJ, Winkler I, et al. G-CSF potently inhibits osteoblast activity and CXCL12 mRNA expression in the bone marrow. *Blood* (2005) 106(9):3020–7. doi:10.1182/blood-2004-01-0272
13. Levesque JP, Hendy J, Takamatsu Y, Simmons PJ, Bendall LJ. Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilization induced by GCSF or cyclophosphamide. *J Clin Invest* (2003) 111(2):187–96. doi:10.1172/JCI13994
14. Kim HK, De La Luz Sierra M, Williams CK, Gulino AV, Tosato G. G-CSF down-regulation of CXCR4 expression identified as a mechanism for mobilization of myeloid cells. *Blood* (2006) 108(3):812–20. doi:10.1182/blood-2005-10-4162
15. Eash KJ, Means JM, White DW, Link DC. CXCR4 is a key regulator of neutrophil release from the bone marrow under basal and stress granulopoiesis conditions. *Blood* (2009) 113(19):4711–9. doi:10.1182/blood-2008-09-177287
16. Hu L, Huang W, Hjort E, Eklund EA. Increased Fanconi C expression contributes to the emergency granulopoiesis response. *J Clin Invest* (2013) 123(9):3952–66. doi:10.1172/JCI69032
17. Hu L, Huang W, Hjort EE, Bei L, Platanias LC, Eklund EA. The interferon consensus sequence binding protein (Icsbp/Irf8) is required for termination of emergency granulopoiesis. *J Biol Chem* (2016) 291(8):4107–20. doi:10.1074/jbc.M115.681361
18. Li Q, Xi S, Sun W, Abdel-Azim H, Lou S, Zhu H, et al. Am80-GCSF synergizes myeloid expansion and differentiation to generate functional neutrophils that reduce neutropenia-associated infection and mortality. *EMBO Mol Med* (2016) 8(11):1340–59. doi:10.15252/emmm.201606434
19. Magnusson M, Brun AC, Miyake N, Larsson J, Ehinger M, Bjornsson JM, et al. HOXA10 is a critical regulator for hematopoietic stem cells and erythroid/megakaryocyte development. *Blood* (2007) 109(9):3687–96. doi:10.1182/blood-2006-10-054676
20. Buske C, Fearing-Buske M, Antonchuk J, Roston P, Hogge DE, Eaves CJ, et al. Overexpression of HOXA10 perturbs human lymphomyelopoiesis in vitro and in vivo. *Blood* (2001) 97(8):2286–92. doi:10.1182/blood.V97.8.2286
21. Konieczna IM, DeLuca TA, Eklund EA, Miller WM. Hoxa10 null animals exhibit reduced platelet biogenesis. *Br J Haematol* (2016) 173(2):303–13. doi:10.1111/bjh.13949
22. Eklund EA, Goldenberg I, Lu Y, Andrejcz J, Kakar R. SHP1 protein-tyrosine phosphatase regulates HoxA10 DNA binding and transcriptional repression activity in undifferentiated myeloid cells. *J Biol Chem* (2002) 277(39): 36878–88. doi:10.1074/jbc.M203917200
23. Eklund EA, Jalava A, Kakar R. Tyrosine phosphorylation of HoxA10 decreases DNA binding and transcriptional repression during interferon gamma-induced differentiation of myeloid leukemia cell lines. *J Biol Chem* (2000) 275(26):20117–26. doi:10.1074/jbc.M907915199
24. Lu Y, Goldenberg I, Bei L, Andrejcz J, Eklund EA. HoxA10 represses gene transcription in undifferentiated myeloid cells by interaction with histone
deacetylase 2. J Biol Chem (2003) 278(48):47792–802. doi:10.1074/jbc.M305885200
25. Krzysztof S, Zhu C, Lu YE, Eklund EA. HoxA10 represses transcription of the gene encoding p67phox in phagocytic cells. J Immunol (2005) 175(8):5269–79. doi:10.4049/jimmunol.175.8.5269
26. Wang H, Lindsey S, Konieczna I, Bei L, Horvath E, Huang W, et al. Constitutive active SHP2 cooperates with HoxA10 overexpression to induce acute myeloid leukemia. J Biol Chem (2009) 284(4):2549–67. doi:10.1074/jbc.M804074200
27. Bei L, Shah C, Wang H, Huang W, Platianis LC, Eklund EA. Regulation of CDX4 gene transcription by HoxA9, HoxA10, the Mll-EIl oncogene and Shp2 during leukemogenesis. Oncogenesis (2014) 3:e135. doi:10.1038/oncsci.2014.49
28. Kroon E, Krosl J, Thorsteinsdottir U, Baban S, Buchberg AM, Sauvageau G. Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b. EMBO J (1998) 17(13):3714–25. doi:10.1093/emboj/17.13.3714
29. Li Z, Luo RT, Mi S, Sun M, Chen P, Bao J, et al. Consistent deregulation of and C/EBPbeta isoform expression. Genes Dev (2000) 14(15):1920–32. doi:10.1101/gad.14.15.1920
30. Anagnostopoulos I, Zhang P, Lu YE, Eklund GA. HoxA10 downregulates C/EBPbeta. J Biol Chem (2016) 291(37):21822–37. doi:10.1074/jbc.M116.742967
31. Chieh W, Lin J, Lin H, Juan S, Niu S, Chen P, et al. Genome-wide analysis of C/EBPalpha and C/EBPbeta expression in human leukemia. Oncogene (2008) 27(17):2285–96. doi:10.1038/onc.2007.490
32. Wang H, Bei L, Shah C, Lu YE, Eklund EA. HoxA10 overexpression cooperates with cytokines to enhance CD34+ progenitor cell colony formation. Leuk Res (2016) 41:24–33. doi:10.1016/j.leukres.2015.12.005
33. Holtschke T, Lohler J, Kanno Y, Fehr T, Giese N, Rosenbauer F, et al. Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein alpha-deficient mice. Proc Natl Acad Sci U S A (1997) 94(2):569–74. doi:10.1073/pnas.94.2.569
34. Zhang P, Iwasaki-Araji J, Iwasaki H, Fenyus ML, Dayaram T, Owens BM, et al. Enhancement of hematopoietic stem cell repopulating capacity and self-renewal in the absence of the transcription factor C/EBP alpha. Immunity (2004) 21(6):853–63. doi:10.1016/j.immuni.2004.11.006
35. Dror N, Rave-Harel N, Burchert A, Azriel A, Tamura T, Tailor P, et al. Interferon regulatory factor-8 is indispensable for the expression of promyelocytic leukemia and the formation of nuclear bodies in myeloid cells. J Biol Chem (2007) 282(8):5633–40. doi:10.1074/jbc.M607825200
36. Burchett A, Cai D, Hofbauer LC, Samuelsson MK, Slater EP, Duyster J, et al. Interferon consensus sequence binding protein (ICSBP, IRF-8) antagonizes BCR/ABL and down-regulates bcl-2. Blood (2004) 103(9):3480–9. doi:10.1182/blood-2003-08-26654
37. Holtschke T, Lohler J, Ianoz F, Fehr T, Giese N, Rosenbauer F, et al. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell (1997) 87(2):307–17. doi:10.1016/S0092-8674(00)81348-3
38. Taniguchi T, Ogasawara K, Takaoka A, Tanaka N. IRF family of transcription factors as regulators of host defense. Annu Rev Immunol (2001) 19:623–55. doi:10.1146/annurev.immunol.19.1.623
39. Zhao J, Kong HJ, Li H, Huang B, Yang M, Zhu C, et al. IRF-8/interferon (IFN) consensus sequence binding protein is involved in toll-like receptor (TLR) signaling and contributes to the cross-talk between TLR and IFN-gamma signaling pathways. J Biol Chem (2006) 281(15):10073–80. doi:10.1074/jbc.M507788200
40. Tsujimura H, Tamura T, Gongora C, Alberti J, Reis e Sousa C, Sher A, et al. ICSBP/IRF-8 retrovirus transduction rescues dendritic cell development in vitro. Blood (2003) 101(3):961–9. doi:10.1182/blood-2002-05-1327
41. Masumi A, Tamaoki S, Wang IM, Ozato K, Komuro K. IRF-8/ICSBP/IRF-8 retrovirus transduction rescues dendritic cell development in vitro. Blood (2003) 101(3):961–9. doi:10.1182/blood-2002-05-1327
42. Asahina M, Tamaoki S, Wang IM, Ozato K, Komuro K. IRF-8/ICSBP and IRF-1 cooperatively stimulate mouse IL-12 promoter activity in macrophages. FEBS Lett (2002) 531(2):348–53. doi:10.1016/S0014-5793(02)00556-1
43. Tsujimura H, Tamura T, Gongora C, Yoshida A, Ishii K, Kinman DM, et al. Toll-like receptor 9 signaling activates NF-kappaB through IFN regulatory factor-8/IFN consensus sequence binding protein in dendritic cells. J Immunol (2004) 172(12):6828–70. doi:10.4049/jimmunol.172.11.6820
44. Yanez A, Ng MY, Hassanazadeh-Kiabi N, Goodridge HS. IRF8 acts in lineage-committed rather than oligopotent progenitors to control neutrophil vs monocyte production. Blood (2015) 125(9):1452–9. doi:10.1182/blood-2014-09-600833
45. Tsujimura H, Nagamasa-Inoue T, Tamura T, Ozato K. IFN consensus sequence binding protein/IFN regulatory factor-8 guides bone marrow progenitor cells toward the macrophage lineage. J Immunol (2002) 169(3):1261–9. doi:10.4049/jimmunol.169.3.1261
46. Kurotaki D, Tamura T. Transcriptional and epigenetic regulation of innate immune cell development by the transcription factor, interferon regulatory factor-8. J Interferon Cytokine Res (2016) 36(7):433–41. doi:10.1089/jir.2015.0138
47. Schmidt M, Nagel S, Proba J, Thiede C, Ritter M, Waring JF, et al. Lack of interferon consensus sequence binding protein (ICSBP) transcripts in human myeloid leukemias. Blood (1998) 91(1):22–9.
48. Schmidt M, Hochhaus A, Nitsche A, Hehlmann R, Neubauer A. Expression of nuclear transcription factor interferon consensus sequence binding protein in chronic myeloid leukemia correlates with pretreatment risk features and cytogenetic response to interferon-alpha. Blood (2001) 97(11):3648–50. doi:10.1182/blood.V79.11.3648
49. Huang W, Bei L, Eklund EA. Fas-associated phosphatase 1 mediates Fas resistance in myeloid progenitor cells expressing the Bcr-abl oncogene. Leuk Lymphoma (2013) 54(3):619–30. doi:10.1080/10428194.2012.720979
62. Huang W, Zhou W, Saberwal G, Konieczna I, Horvath E, Katsoulidis E, et al. Interferon consensus sequence binding protein (ICSBP) decreases beta-catenin activity in myeloid cells by repressing GAS2 transcription. Mol Cell Biol. 2010(30):4575–94. doi: 10.1128/MCB.01595-09.

63. Oda A, Wakao H, Fujita H. Calpain is a signal transducer and activator of transcription (STAT) 3 and STAT5 promoter. Blood (2002) 99(5):1850–2. doi: 10.1182/blood.V99.5.1850.

64. Hjort EE, Huang W, Hu L, Eklund EA. Bcr-abl regulates Stat5 through Shp2, the interferon consensus sequence binding protein (Icsbp/Irf8), growth arrest specific 2 (Gas2) and calpain. Oncotarget (2016) 7(47):77635–50. doi: 10.18632/oncotarget.12749.

65. Chen Y, Peng C, Abraham SA, Shan Y, Guo Z, Desouza N, et al. Archichodamine 15-lipoxygenase is required for chronic myeloid leukemia stem cell survival. J Clin Invest (2014) 124(9):3847–62. doi: 10.1172/JCI66129.

66. Saberwal G, Horvath E, Hu L, Zhu C, Hjort E, A Song G, et al. PU.1 cooperates with IRF4 and IRF8 to suppress pre-B-cell leukemia. Leukemia (2016) 30(6):1375–87. doi: 10.1038/leu.2016.27.

67. Akbarzadeh S, Ward AC, McPhee DO, Alexander WS, Lieschke GJ, Layton JE. Tyrosine residues of the granulocyte colony-stimulating factor receptor are required for transcriptional activation and differentiation signals in murine bone marrow cells. Blood (2002) 99(3):879–87. doi: 10.1182/blood.V99.3.879.

68. Tian SS, Lamb P, Seidel HM, Stein RB, Rosen J. Rapid activation of the STAT3 transcription factor by granulocyte colony-stimulating factor. Blood (1994) 84(6):1760–4.

69. Szekely K, Biethahn S, Wilde S, Hiddemann W, Alves F. Constitutive STAT3 is a negative regulator of granulopoiesis but is not required for emergency granulopoiesis during granulocyte and macrophage differentiation. Exp Hematol (2008) 36(7):786–98. doi: 10.1016/j.exphem.2008.02.008.

70. Shah CA, Broglie L, Hu L, Bei L, Huang W, Dressler DB, et al. STAT3 and interferon consensus sequence binding protein (ICSBP) decreases beta3 integrin expression, cell migration, and induction of apoptosis by a novel small-molecule Stat3 inhibitor. Cytometry B Clin Cytom. (2010) 78(7):714–25. doi: 10.1002/cyto.20659.

71. Spiekermann K, Biethahn S, Wilde S, Hiddemann W, Alves F. Constitutive STAT3 controls myeloid progenitor growth during emergency granulopoiesis. Mol Cell (2014) 55(2):295–306. doi: 10.1016/j.molcel.2014.03.014.

72. Croker BA, Mielke LA, Wormald S, Metcalf D, Kiu H, Alexander WS, et al. SOCS3 maintains the specificity of biological responses to cytokine signals during granulocyte and macrophage differentiation. Exp Hematol (2008) 36(7):786–98. doi: 10.1016/j.exphem.2008.02.008.

73. Hasnati A, Shimoda K, Kamekazi K, Haro T, Kakumitsu H, Shide K, et al. Signal transducers and activators of transcription 3 augments the transcriptional activity of CCAAT/enhancer-binding protein alpha in granulocyte colony-stimulating factor signaling pathway. J Biol Chem (2005) 280(13):12621–9. doi: 10.1074/jbc.M408442200.

74. Kimura A, Kinjo Y, Matsumura Y, Mori H, Mashima R, Harada M, et al. SOCS5 is a physiological negative regulator for granulopoiesis and granulocyte colony-stimulating factor receptor signaling. J Biol Chem (2004) 279(8):6905–10. doi: 10.1074/jbc.C300496200.

75. Schuringa JJ, Wierenga AT, Kruijver W, Vellenga E. Constitutive Stat3, Ty705, and Sert727 phosphorylation in acute myeloid leukemia cells caused by the autocrine secretion of interleukin-6. Blood (2000) 95(12):3765–70.

76. Panopoulos AD, Zhang L, Snow JW, Jones DM, Smith AM, El Kasmi KC, et al. STAT3 governs distinct pathways in emergency granulopoiesis and mature neutrophils. Blood (2006) 108(12):3682–90. doi: 10.1182/blood-2006-02-03012.

77. Hwang S, DiStefano PS, Thery C, Juneja J, Zampaglione N, Wang Y, et al. Protein tyrosine phosphatase Meg2 dephosphorylates signal transducer and activator of transcription 3 and suppresses tumor growth in breast cancer. Breast Cancer Res (2012) 14(2):R36. doi: 10.1186/bcr3134.

78. Hwang S, DiStefano PS, Thery C, Juneja J, Zampaglione N, Wang Y, et al. Protein tyrosine phosphatase Meg2 dephosphorylates signal transducer and activator of transcription 3 and suppresses tumor growth in breast cancer. Breast Cancer Res (2012) 14(2):R36. doi: 10.1186/bcr3134.

79. Hwang S, DiStefano PS, Thery C, Juneja J, Zampaglione N, Wang Y, et al. Protein tyrosine phosphatase Meg2 dephosphorylates signal transducer and activator of transcription 3 and suppresses tumor growth in breast cancer. Breast Cancer Res (2012) 14(2):R36. doi: 10.1186/bcr3134.

80. Hwang S, DiStefano PS, Thery C, Juneja J, Zampaglione N, Wang Y, et al. Protein tyrosine phosphatase Meg2 dephosphorylates signal transducer and activator of transcription 3 and suppresses tumor growth in breast cancer. Breast Cancer Res (2012) 14(2):R36. doi: 10.1186/bcr3134.

81. Hwang S, DiStefano PS, Thery C, Juneja J, Zampaglione N, Wang Y, et al. Protein tyrosine phosphatase Meg2 dephosphorylates signal transducer and activator of transcription 3 and suppresses tumor growth in breast cancer. Breast Cancer Res (2012) 14(2):R36. doi: 10.1186/bcr3134.
101. Levy DE, Darnell JE Jr. Stats: transcriptional control and biological impact. *Nat Rev Mol Cell Biol* (2002) 3(9):651–62. doi:10.1038/nrm9099

102. Schwaller J, Parganas E, Wang D, Cain D, Aster JC, Williams JR, et al. STAT5 is essential for the myelo- and lymphoproliferative disease induced by TEL/ AK2. *Mol Cell* (2000) 6(3):693–704. doi:10.1016/S1097-2765(00)00067-8

103. Liu S, Walker SR, Nelson EA, Cerulli R, Xiang M, Toniole PA, et al. Targeting STAT5 in hematologic malignancies through inhibition of the bromodomain and extra-terminal (BET) bromodomain protein BRD2. *Mol Cancer Ther* (2014) 13(5):1194–205. doi:10.1158/1535-7163.MCT-13-0341

104. Snow JW, Abraham N, Ma MC, Abbey NW, Herndier B, Goldsmith MA. STAT5 promotes multilineage hematolymphoid development in vivo through effects on early hematopoietic progenitor cells. *Blood* (2002) 99(1):95–101. doi:10.1182/blood.V99.1.95

105. Skokowa J, Cario G, Uenalan M, Schambach A, Gernsheim J, Mattern K, et al. LEF-1 is crucial for neutrophil granulocytopoiesis and its expression is severely reduced in congenital neutropenia. *Nat Med* (2006) 12(10):1191–7. doi:10.1038/nm1474

106. Michl J, Zimmer J, Tarsounas M. Interplay between Fanconi anemia and homologous recombination pathways in genome integrity. *EMBO J* (2016) 35(9):909–23. doi:10.15252/embj.201693860

107. Zhang J, Walter JC. Mechanism and regulation of incisions during DNA interstrand cross-link repair. *DNA Repair (Amst)* (2014) 19:135–42. doi:10.1016/j.dnarep.2013.03.018

108. Howlett NG, Taniguchi T, Olson S, Cox B, Waisfisz Q, De Die-Smulders C, et al. Biallelic inactivation of BRCA2 in Fanconi anemia. *Science* (2002) 297(5581):606–9. doi:10.1126/science.1073834

109. Kelland L. The resurgence of platinum-based cancer chemotherapy. *Nat Rev Cancer* (2007) 7(8):573–84. doi:10.1038/nrc2167

110. Zhang J, Dewar JM, Budzowska M, Mottenko A, Cohn MA, Walter JC. DNA interstrand cross-link repair requires replication-fork convergence. *Nat Struct Mol Biol* (2015) 22(3):242–7. doi:10.1038/nsmb.2956

111. Higgs MR, Reynolds JJ, Winczura A, Blackford AN, Borel V, Miller ES, et al. DNA DSB1 is required to suppress deleterious resection of stressed replication forks. *Mol Cell* (2015) 59(3):462–77. doi:10.1016/j.molcel.2015.06.007

112. Long DT, Raschle M, Joukov V, Walter JC. Mechanism of RAD51-dependent DNA interstrand cross-link repair. *Science* (2011) 333(6038):84–7. doi:10.1126/science.1204258

113. Kennedy RD, D’Andrea AD. The Fanconi anemia/BRCA pathway: new faces in the crowd. *Genes Dev* (2005) 19(24):2925–40. doi:10.1101/gad.1370505

114. Bagby GC Jr. Genetic basis of Fanconi anemia. *Curr Opin Hematol* (2003) 10(1):68–76. doi:10.1097/00002672-200301000-00011

115. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia. *Blood* (2003) 101(3):822–6. doi:10.1182/blood-2002-05-1498

116. Quentin S, Cuccuini W, Ceccaldi R, Nibourel O, Ponzarre C, Pages MP, et al. Myelodysplasia and leukemia of Fanconi anemia are associated with a specific pattern of genomic abnormalities that includes cryptic RUNX1/AML1 lesions. *Blood* (2011) 117(15):e161–70. doi:10.1182/blood-2010-09-308726

117. Hira A, Yabe H, Yoshida K, Okuno Y, Shiroya H, Chiba K, et al. Variant ALDH2 is associated with accelerated progression of bone marrow failure in Japanese Fanconi anemia patients. *Blood* (2013) 122(18):3206–9. doi:10.1182/blood-2013-06-70962

118. Ceccaldi R, Parmar K, Mouly E, Delord M, Kim JM, Regairaz M, et al. Bone marrow failure in Fanconi anemia is triggered by an exacerbated p53/p21 DNA damage response that impairs hematopoietic stem and progenitor cells. *Cell Stem Cell* (2012) 11(1):36–49. doi:10.1016/j.stem.2012.03.013

119. Du W, Amarachinthia S, Ereden O, Wilson A, Pang Q. The Fanconi anemia pathway controls oncogenic response in hematopoietic stem and progenitor cells by regulating PRMT5-mediated p53 arginine methylation. *Oncotarget* (2016) 7(37):60005–20. doi:10.18632/oncotarget.11088

120. Cerahona D, Sun Z, Nalepa G. Leukemia and chromosomal instability in aged Fanc−/− mice. *Exp Hematol* (2016) 44(5):352–7. doi:10.1016/j.exphem.2016.01.009

121. Tischkowitz M, Ameziane N, Waisfisz Q, De Winter JP, Harris R, Taniguchi T, et al. Bi-allelic silencing of the Fanconi anaemia gene FANC-F in acute myeloid leukaemia. *Br J Haematol* (2003) 123(3):469–71. doi:10.1046/j.1365-2457.2003.04640.x

122. Kottemann MC, Smogorzewska A. Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature* (2013) 493(7452):356–63. doi:10.1038/nature11863

123. Seal S, Thompson D, Renwick A, Elliott A, Kelly P, Barfoot R, et al. Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. *Nat Genet* (2006) 38(11):1239–41. doi:10.1038/ng1902

124. Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, et al. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat Genet* (2007) 39(2):165–7. doi:10.1038/ng1959

125. Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, et al. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat Genet* (2007) 39(2):165–7. doi:10.1038/ng1959

126. Meindl A, Hellebrand H, Wiek C, Erven V, Wappenschmidt B, Niederacher D, et al. Germline mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. *Nat Genet* (2010) 42(5):410–4. doi:10.1038/ng1959

127. Deans AF, West SC. DNA interstrand crosslink repair and cancer. *Nat Rev Cancer* (2011) 11(7):467–80. doi:10.1038/nrc3088

128. Schlaicher K, Wu H, Jasinska A. A distinct replication fork protection pathway connects Fanconi anemia tumor suppressors to RAD51/BRCA1/2. *Cancer Cell* (2012) 22(1):106–16. doi:10.1016/j.ccell.2012.05.015

129. Schlaicher K, Christ N, Siaud N, Egashira A, Wu H, Jasinska A. Double-strand break repair-independent role for BRCA2 in blocking stalled replication fork degradation by MRE11. *Cell* (2011) 145(4):529–42. doi:10.1016/j.cell.2011.03.041

130. Howlett NG, Taniguchi T, Gurkin SG, D’Andrea AD, Glover TW. The Fanconi anemia pathway is required for the DNA replication stress response and for the regulation of common fragile site stability. *Hum Mol Genet* (2005) 14(5):693–701. doi:10.1093/hmg/ddi065

131. Konieczna I, Horvath E, Wang H, Lindsey S, Saberwal G, Bei L, et al. Constitutive activation of SH2P in mice cooperates with IC5BP deficiency to accelerate progression to acute myeloid leukemia. *J Clin Invest* (2008) 118(3):853–67. doi:10.1172/JCI33742

132. Williams MD, Braun LA, Cooper LM, Johnston J, Weiss RV, Qualy RL, et al. Replication fork degradation by MRE11. *Nat Genet* (2006) 38(11):1239–41. doi:10.1038/ng1902

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