New insights into the pathomechanism of cyclic neutropenia

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Cyclic neutropenia (CyN) is a hematologic disorder in which peripheral blood absolute neutrophil counts (ANCs) show cycles of approximately 21-day intervals. The majority of CyN patients harbor ELANE mutations, but the mechanism of ANC cycling is unclear. We performed analysis of bone marrow (BM) subpopulations in CyN patients at the peak and the nadir of the ANC cycle and detected high proportions of BM hematopoietic stem cells (HSCs) and hematopoietic stem and progenitor cells (HSPCs) at the nadir of the ANC cycle, as compared with the peak. BM HSPCs produced fewer granulocyte colony-forming unit colonies at the ANC peak. To investigate the mechanism of cycling, we found that mRNA expression levels of ELANE and unfolded protein response (UPR)–related genes (ATF6, Bip (HSPA5), CHOP (DDIT3), and PERK (EIF2AK3)) were elevated, but antiapoptotic genes (Bcl-2 (BCL2) and bcl-xL (BCL2L1)) were reduced in CD34+ cells tested at the ANC nadir. Moreover, HSPCs revealed increased levels of reactive oxygen species and gH2AX at the ANC nadir. We suggest that in CyN patients, some HSPCs escape the UPR-induced endoplasmic reticulum (ER) stress and proliferate in response to granulocyte colony-stimulating factor (G-CSF) to a certain threshold at which UPR again affects the majority of HSPCs. There is a cyclic balance between ER stress–induced apoptosis of HSPCs and compensatory G-CSF–stimulated HSPC proliferation followed by granulocytic differentiation.

Keywords: cyclic neutropenia (CyN); UPR; ELANE mutations; ROS; DNA damage

Introduction

Homeostasis of the production of neutrophils in the bone marrow (BM) is strictly regulated by the balance of activators, such as granulocyte colony-stimulating factor (G-CSF) or granulocyte-macrophage colony-stimulating factor (GM-CSF), and by the feedback of the number of neutrophils in the blood, for example, by currently unidentified molecular sensors for neutrophils. Severe congenital neutropenias (CNs) are a heterogeneous group of rare diseases characterized by impaired maturation of neutrophil granulocytes. The most frequent pathogenic defects are autosomal dominant mutations in ELANE, which encodes for neutrophil elastase (NE). Patients harboring ELANE mutations suffer from cyclic neutropenia (CyN) that is characterized by periodically oscillating numbers of blood neutrophils, monocytes, platelets, reticulocytes, and lymphocytes, usually with a 21-day periodicity. Intriguingly, patients with CyN can harbor ELANE mutations at the same amino acid positions as patients with CN. CyN has a lower risk of progression to myelodysplastic syndrome and acute myeloid leukemia (AML), milder morbidity, and requirement of less G-CSF to achieve a median of >1000 neutrophils/μL. However, when neutrophil counts are <500/μL for more

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than 7 days within the cycle, patients regularly have painful mouth ulcers, upper respiratory tract infections, skin abscesses, and suffer from malaise.

The central pathophysiological mechanism of CyN is the failure of the BM to maintain consistent production of mature neutrophils. Severe neutropenia recurs when the BM supply is exhausted. This process then repeats regularly. Researchers have puzzled over the mechanisms of the cycling of neutrophils; several mathematicians have proposed explanations. The first model by Michael Mackey postulates that the production of neutrophils is governed by long-range stimulatory factors in a long feedback loop that has a built-in time delay in the maturation of promyelocytes to fully differentiated neutrophils.\(^9,10\) Cell loss early in this process (at the promyelocyte stage) would be associated with oscillations in blood neutrophil counts. Furthermore, in case of the severe cell loss, there might be no apparent oscillations, because neutrophil counts would remain continually extremely low. Intriguingly, treatment with G-CSF in patients with CyN does not abrogate cycling, but increases the absolute neutrophil count (ANC), shortens the cycle periodicity from the usual 21 days to about 14 days, and prevents serious infections.\(^3\) Moreover, administration GM-CSF did not affect neutrophil counts in CyN patients\(^11\) as has also been reported for CN patients.\(^14,18,19\)

CyN is a stem cell disorder since it can be transferred by allogeneic marrow grafting: the allogeneic BM transplantation from a sibling suffering from CyN into a patient with relapsed acute lymphoblastic leukemia led to CyN in the recipient.\(^13\)

Two theories describing how \textit{ELANE} mutations affect NE functions have been proposed. We and others reported that \textit{ELANE} mutations cause an unfolded protein response (UPR) in the endoplasmic reticulum (ER), leading to the activation of ER proteins, such as ATF6, PERK, ATF4, and so on, and subsequent ER stress.\(^14-17\) In the other theory, mutant NE is mistrafficked.\(^14,18,19\) Tidwell \textit{et al.} suggested that Kozak sequence \textit{ELANE} mutations occur in CyN patients, resulting in the production of both full-length protein and reduced levels of the amino-terminally truncated isoforms. Mutated full-length protein is associated with UPR. Lower expression of the shortened isoform in CyN, as compared with CN patients, correlates with what might be considered as milder disease.\(^20\)

Van Galen \textit{et al.} reported a link between UPR signaling and human HSPC function.\(^21\) They pointed to the elimination of individual HSPCs after stress and damage as a paradigm of how the stem-cell pool maintains integrity, thereby ensuring long-term tissue maintenance.

In the present work, we aimed to investigate the role of the UPR caused by mutated elastase protein and the subsequent ER stress and reactive oxygen species (ROS) in the course of ANC cycles, and we present a hypothesis that UPR might be involved in the pathomechanism of cycling hematopoiesis.

**Materials and methods**

**Patients**

Severe CN and CyN patients harboring \textit{ELANE} mutations (\textit{ELANE-CN} and \textit{ELANE-CyN}, respectively) were used in the study. BM samples from patients were collected in association with an annual follow-up recommended by the Severe Chronic Neutropenia International Registry. Study approval was obtained from the ethical review board of the Medical Faculty of the University of Tübingen. Informed written consent was obtained from all participants of this study.

**FACS analysis of surface marker expression, gH2AX, and ROS**

BM mononuclear cells were washed with phosphate-buffered saline (PBS) and stained with corresponding surface marker antibodies in PBS containing 2% fetal bovine serum (FBS) and 0.02% sodium azide. For surface marker analysis (panel adapted from van Galen \textit{et al.}\(^21\)), the following antibodies were used: mouse anti-human CD38 (BD, #563964), mouse anti-human CD34 (BD, #348811), rat anti-human CD49f (BD, #563271), mouse anti-human CD90 (BD, #562685), mouse anti-human CD45RA (BD, #560673), mouse anti-human CD10 (BD, #563734), and mouse anti-human CD135 (BD, #564708). For the analysis of intracellular gH2AX protein, cells were subsequently permeabilized and fixed using the IntraSure\textsuperscript{TM} Kit (BD #641776), followed by incubation with mouse anti-human gamma gH2AX (BD, #560445) antibody. For the analysis of ROS, cells were incubated with 20 \textmu M 2’,7’-dichlorofluorescin diacetate (DCFH-DA) (Sigma-Aldrich/Merck, #D6883) for 10 min at 37°C after staining with surface marker antibodies.
Cell purification and separation
We isolated BM mononuclear cells by Ficoll-Hypaque gradient centrifugation (Amersham Biosciences) and positively selected BM CD34⁰ cells by sequential immunomagnetic labeling with anti-CD34⁺ magnetic cell-sorting beads (Miltenyi Biotech). We assessed cell viability by trypan blue dye exclusion. The purity of the sorted CD34⁺ cells was more than 95% as determined by FACS analysis.

Colony-forming unit assay
CD34⁺ cells were resuspended in IMDM supplemented with 2% FBS (Stemcell Technologies, #07700) and enriched MethoCult™ (Stemcell Technologies, #H4435). The cell suspension was plated on 3.5 cm dishes (1 × 10⁵ cells/dish) for 14 days.

mRNA isolation and cDNA amplification
RNA was isolated using the RNeasy® Mini Kit (Qiagen, #74106) according to the manufacturer’s instructions. cDNA was amplified using the Ovation® Pico WTA system 2 (NuGEN, San Carlos, CA), which amplifies cDNA out of a small quantity of RNA.

qRT-PCR analysis
The mRNA expression levels were measured using the SYBR Green quantitative PCR kit (Roche, #04887352001). Target gene mRNA expression was normalized to β-actin and GAPDH, and is presented as arbitrary units. Primer sequences are as follows: ELANE_F: TCT TCA GGT TTC AGG CTC AAC A, ELANE_R: AGC GTT GGA TGA TAG AGT CGA TC; β-actin_F: TTC CTG GGC ATG GAG TC, β-actin_R: CAG GTC TTT GCG GAT GTC; CHOP_F: GCC TTT CTC CTT TGG GAC ACT GTC CAG C, CHOP_R: CTC GGC GAG TCG CCT CTA CTT CCC; BiP_F: AGG ACA AGA AGG AGG AGG TGG, BiP_R: GGT TGG AGG TGA GCT GGT TC; ATF6_F: TGC TCT CTT TGC TGA ACT CGG, ATF6_R: GCT GGA GAA AGT GGC TGA GGT; PERK_F: AGG GGC ACT CCT TTG AAC TT, PERK_R: AT'T TGC TAC TGG TGC GCT TG; BCL2_F: TAG TTG TTG TGC TCG CCA GAA CG, BCL2_R: GGA CTC AAC ACC TGA GGC CA; BCL-XL_F: GGG TTC CCT TTC CTT CCA TC, BCL-XL_R: AGT GGC CCT AAA TGG CTC T.

Statistics
Statistical analysis was performed using a two-sided unpaired Student’s t-test for the evaluation of differences in mean values between groups.

Results
CyN patients have cycling ANC in the peripheral blood and less severe disease than CN patients
Representative graphs of ANCs evaluated in the peripheral blood (PB) of CyN patients demonstrated cycling of neutrophil granulocytes (Fig. 1A). Even before initiation of G-CSF therapy, ANCs show low amplitude cycles (data not shown). After induction of G-CSF therapy, ANC cycles have much higher amplitude and are shorter (Fig. 1A).

The course of disease in CyN patients is less severe. The median therapeutic G-CSF dosages required to achieve >1000 neutrophils/μL in PB was 4.9 μg/kg/day for 72 ELANE-CN patients and 1.5 μg/kg/day for 24 ELANE-CyN patients. The cumulative incidence of developing AML is approximately 20% in ELANE-CN patients and approximately 1% in ELANE-CyN patients (Table 1). ELANE mutations within the same amino acid of the NE protein can occur in CN and CyN patients,

| Patients         | G-CSF dose | AML   |
|------------------|------------|-------|
| ELANE-CN (n = 72)| 4.9 μg/kg/day | 20%   |
| ELANE-CyN (n = 24) | 1.5 μg/kg/day | 1%    |

Table 1. Clinical data on the median G-CSF dosages required to reach ANC over 1000/mL, and leukemia development in ELANE-CN and ELANE-CyN patients
and there are no mutually exclusive ELANE mutations for one or the other disorder.⁷

**Elevated proportion of HSCs in the BM of CyN patients isolated at the stage of the nadir of PB PMNs**

To investigate the BM composition in CyN patients at different stages of the PB ANC cycle, we performed FACS analysis using the multicolor antibody panel established in the laboratory of John Dick. BM mononuclear cells were isolated from three CyN patients at the nadir of PB ANC (ANC nadir) and from three CyN patients at the maximum of PB ANC counts (ANC peak). We found markedly elevated proportions of HSPCs, CD34⁺CD38⁺ progenitor cells, and common myeloid progenitors (CMPs) at the ANC nadir, as compared with the ANC peak (Fig. 2A). Compensatorily, the percentage of multilymphoid progenitors (MLPs), megakaryocyte-erythroid progenitors (MEPs), and B/NK progenitors was increased at the ANC peak or unchanged between two stages of the ANC cycle.

![Figure 2](image-url)

**Figure 2.** Increased percentage of HSPC populations in the bone marrow of CyN patients at the ANC nadir. (A) The percentage of CD34⁺CD38⁻CD45RA⁻CD90⁻CD49f⁺ HSCs, CD34⁺CD38⁺ hematopoietic progenitor cells, CD34⁺CD38⁺CD45RA⁻CD10⁻CD135⁺ CMPs, CD34⁺CD38⁻CD45RA⁺CD90⁻/lo MLPs, CD34⁺CD38⁺CD45RA⁻CD10⁻CD135⁻ MEPs, and CD34⁺CD38⁺CD45RA⁺CD10⁺ B/NK cells in the bone marrow of CyN patients was assessed by multicolor FACS according to the panel described in the Materials and Methods section (CyN at the ANC nadir, n = 3; CyN at the ANC peak, n = 3). Data represent means ± SD; *P < 0.05; **P < 0.01. (B) CD33 expression was evaluated in the bone marrow of two CyN patients at the ANC nadir and ANC peak by FACS. Data represent means ± SD; *P < 0.05. (C) CFU assay of the bone marrow cells of two CyN patients. Data represent means ± SD from triplicates of each patient; *P < 0.05 compared with ANC nadir.
Table 2. Analysis of bone marrow smears of CyN patients

| Monocytes    | BM (ANC peak) | BM (ANC nadir) |
|--------------|---------------|---------------|
| Erythropoiesis | 14.5          | 34.5          |
| Promyelocytes | 5             | 10.5          |
| Myelocytes   | 3             | 3.5           |
| Neutrophils  | 32            | 16            |
| Eosinophils  | 9.5           | 9.5           |
| Lymphocytes  | 20            | 17.5          |
| Monocytes    | 11            | 7             |

(Fig. 2A). Independent FACS analysis also revealed a reduced proportion of CD33\(^{high}\) promyelocytes at the ANC peak, as compared with the ANC nadir (6% versus 47%) (Fig. 2B). Accordingly, HSPCs isolated at the ANC nadir produced much more granulocyte colony-forming units (CFU-G), as compared with cells isolated at the ANC peak (Fig. 2C). Morphological examination of myeloid precursor cells (e.g., promyelocytes) in BM smears confirmed this observation (Table 2).

**Elevated mRNA expression levels of ELANE and UPR-related genes at the nadir of the ANC cycle in CyN patients**

We detected increased ELANE mRNA levels at the ANC nadir, as compared with the ANC peak (Fig. 3A). In line with these observations, mRNA expression levels of key effectors of the UPR, such as ATF6 (activating transcription factor 6), the endoplasmic reticulum chaperone BIP (binding-immunoglobulin protein or HSP70 family protein 5, HSPA5), CHOP (C/EBP homologous protein), and PERK (protein kinase R (PKR)-like endoplasmic reticulum kinase), were elevated in BM CD34\(^{+}\) HSPCs of CyN patients isolated at the ANC nadir, as compared with the peak of the ANC cycle (Fig. 3B).

**ROS and gH2AX levels are upregulated in the BM HSCs of CyN patients at the nadir of the PMN cycle**

We further evaluated whether elevated UPR may induce ROS and DNA damage in the BM hematopoietic cells. We detected elevated ROS and gH2AX in HSCs and HSPCs at the ANC nadir, as compared with the ANC peak (Fig. 4A and B). In addition, we also found reduced mRNA expression of antiapoptotic factors Bcl-2 (BCL2) and bcl-xL (BCL2L1) in CD34\(^{+}\) cells of CyN patients at the ANC nadir, as compared with the ANC peak (Fig. 4C).

**Discussion**

For many decades, researchers have puzzled over the mechanisms of cycling of neutrophils in CyN patients.\(^{3,4,9,22}\) Lei and Mackey postulated that long-range stimulatory factors govern the production of neutrophils by a feedback loop that has a built-in time delay in the maturation of promyelocytes to fully differentiated neutrophils.\(^{9,10}\) Other researchers searched for clock genes responsible for the 21-day cycle of neutrophils or other treatment avenues but have been unsuccessful so far. The availability of recombinant G-CSF for clinical use since 1987\(^{23,24}\) and its successful application to CyN patients changed the outcome for these patients, leading to a normal quality of life.\(^{3,6}\) Intriguingly, the treatment of CyN patients with G-CSF did not abrogate the cycling of neutrophil counts; instead, it increased the amplitude of the neutrophil counts during the cycle, leading to a shorter period of severe neutropenia in each cycle and an increase in the average counts of neutrophils\(^{3}\) (Fig. 1). Finally, G-CSF treatment leads to a shortening of the period of the cycle length from 21 days to approximately 14 days, suggesting that G-CSF is involved in the mechanism of neutrophil cycling. Treatment with GM-CSF did not affect the neutrophil counts of CyN patients.\(^{11}\) The G-CSF–induced shortening of cycles implies that the enhanced G-CSF–mediated production of neutrophils also accelerates the pathomechanism leading to neutrophil cycling. Indeed, if the UPR caused by misfolded mutated elastase protein in the ER and the subsequent ER stress–induced ROS generation is considered as the underlying pathomechanism for the periodic shutdown of neutrophil production, there might be a flip-flop situation between G-CSF–induced differentiation of neutrophils and the destruction of HSCs and progenitor cells by UPR.

One hypothesis is that the inhibition of proliferation and differentiation of HSCs and progenitor cells induced by UPR and ROS in CyN as compared with CN is not complete, so that some cells escape from the damage (escaper cells) and respond to G-CSF with a new generation of HSCs, progenitor cells, and neutrophils. In the CyN situation, this scenario translates to the following: at the ANC nadir, the high levels of UPR and ROS caused by...
the mutated elastase protein lead to damage of the elevated numbers of HSCs and progenitor cells in the BM. However, the already differentiated precursor cells still generate neutrophils until they are exhausted. Therefore, at the peak of ANC, CyN patients have the highest neutrophil counts. The few unaffected and escaping HSCs and progenitor cells respond to G-CSF. At the peak of the cycle, their numbers are still low, but during the remainder of the cycle they proliferate in response to G-CSF to generate increased numbers of HSCs and progenitor cells at the nadir. At the nadir of the cycle, they suffer again from UPR stress (Fig. 5). This is supported by data showing that UPR causing mutated ELANE expression is highest at the nadir. This balance between generation of neutrophils from damage-escaping HSCs and progenitor cells and G-CSF stimulation of proliferation of new HSCs and progenitor cells leads to the cycling of PB neutrophils. This also explains the controversial proportions of progenitor cells and mature neutrophils during the cycle: at the neutrophil

![Figure 3](image)

**Figure 3.** Expression of ELANE and UPR genes is severely increased in CyN CD34+ bone marrow cells at the ANC nadir. mRNA expression levels of ELANE (A) and UPR genes (B) were measured by qRT-PCR in CD34+ bone marrow progenitors of CyN patients isolated at the ANC nadir (n = 3) and at the ANC peak (n = 3). Data represent means ± SD of triplicates; *P < 0.05; **P < 0.01.
Figure 4. Elevated ROS and gH2AX levels as well as diminished expression of bcl-2 are seen in HSCs of CyN patients at the ANC nadir. Bone marrow mononuclear cells of two CyN patients at nadir (n = 2) and peak (n = 2) of ANC, respectively, were stained for surface markers to separate CD34<sup>+</sup>CD38<sup>−</sup>CD45RA<sup>−</sup>CD90<sup>+</sup>CD49f<sup>+</sup> HSCs and CD34<sup>+</sup>CD38<sup>−</sup> HSPCs. ROS (A) and gH2AX (B) were evaluated as described in the Materials and Methods section. Data represent means ± SD of duplicates; *P < 0.05; **P < 0.01. (C) mRNA expression levels of indicated genes were measured by qRT-PCR in CD34<sup>+</sup> bone marrow progenitors of CyN patients isolated at the ANC nadir (n = 2) and at the ANC peak (n = 2). Data represent means ± SD of triplicates; *P < 0.05; **P < 0.01.

nadir—a high proportion of stem and progenitor cells; at the ANC peak—low myeloid progenitor cells. So, cycling neutropenia is characterized by cycling UPR activities and cycling UPR-escaping cells (Fig. 5). UPR is causing loss of stem and progenitor cells, but there are always cells escaping UPR, either preexisting precursor cells at the nadir or stem and progenitor cells later during the cycle.
The so-called “escaper cells” respond to G-CSF to generate either neutrophils between nadir and peak or new stem and progenitor cells during the cycle, reaching a maximum at the nadir (Fig. 5). Other cell lineages, such as erythroid or lymphoid progenitor cells, are not affected by UPR since they are not expressing mutated ELANE. However, the cause of cycling UPR remains to be investigated and is a subject of ongoing research.

UPR also plays an essential role in the regulation of cycling of intestinal epithelial stem cells. The escape of stem cells from mutational damage was also shown for Paneth cells in the intestinal crypt, where these escaping stem cells, which are affected by mitochondrial UPR leading to loss of stemness and cell proliferation, respond to Wnt to reconstitute functional crypts.

Another hypothesis would be that not all HSCs of CyN patients proliferate at similar rates. A report by Foudi et al. assumes a larger population (~80%) of HSCs cycling faster and a smaller population (~20%) of HSCs cycling more slowly. They argue that a proliferation clock for HSCs exists. Another study by Wilson et al. shows that HSCs with the highest self-renewal capacity are kept in a dormant state to preserve their activity, and their stem cell capacity can be reversibly activated in emergency situations, leading to efficient and rapid renewal of blood lineages. The hypothesis of two stem cell compartments is also supported by data from hair follicle cycling and intestinal crypt stem cell cycling. Translating this two stem cell compartment hypothesis to the situation of CyN would mean that the UPR, ROS, and ER stress caused by the mutated elastase protein would affect mainly the fast-proliferating, more mature HSCs at the nadir of the cycle and spare the slow-proliferating, more immature HSCs. This is also supported by data by van Galen et al., namely that the elimination of individual HSCs after stress and damage is a paradigm of how the stem-cell pool maintains integrity, thereby ensuring long-term tissue maintenance. The fast-proliferating HSCs undergo apoptosis, but the already differentiated, more mature myeloid cells, such as, for example, myeloblasts and promyelocytes, derived from the fast-proliferating HSC pool before they die, still differentiate to mature neutrophils, giving rise to the ANC peak values during the time between the nadir and the peak of cycle. At the ANC peak, the low number of slow-proliferating HSC and progenitor cells is only present. Between the peak and the nadir of the cycle, these slow-proliferating HSCs respond to G-CSF and give rise to the fast-proliferating HSCs and progenitor cell pool present at the nadir of the cycle that are again prone to be abrogated by UPR, ER stress, elevated ROS, and apoptosis. This hypothesis is also supported by the fact that the ELANE mutations cause UPR only in the fast-proliferating, more mature HSCs since the expression of elastase protein is restricted to more mature HSCs and progenitor cells. Further studies are needed to confirm one of these hypotheses.

The fact that G-CSF shortens the cycle length supports the notion that it is involved in the mechanism of cycling by accelerating the proliferation of HSCs and progenitor cells during the cycle. The question why patients with CN harboring the same mutations within ELANE do not cycle their neutrophil counts can be explained by the different severity of the two disorders of myelopoiesis. In CN patients, the damage caused by the UPR stress is more severe than in CyN patients so that there is either no UPR stress—escaping HSCs and progenitor cells or no remaining fast-proliferating HSCs that could still differentiate to mature neutrophils, and the treatment with G-CSF supports only differentiation of slow-proliferating HSCs. Our hypothesis regarding the different severities of the underlying disorders of myelopoiesis (CN versus CyN) is further supported by the fact that CN patients need a median of 5 μg/kg/day G-CSF and CyN patients an average of 1.5 μg/kg/day to only achieve a median neutrophil count of more than 1000/μL. Moreover, the risk to develop leukemia...
is much higher in CN patients (∼20%) than in CyN patients (∼1%). Indeed, the risk for leukemia development has been reported to be dependent on the degree of ER stress.32

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Author contributions

K.W., J.S., and P.M. made initial observations, designed the experiments, analyzed the data, supervised experimentation, and wrote the manuscript; P.M. performed the main experiments; K.H. assisted with some FACS experiments; M.K. assisted with mRNA isolation and cDNA amplification; B.F. performed some qPCRs; and S.M.H. and C.Z. provided patient materials and clinical data.

Competing interests

The authors declare no competing interests.

References

1. Welte, K., C. Zeidler & D.C. Dale. 2006. Severe congenital neutropenia. Semin. Hematol. 43:189–195.
2. Skokowa, J., D.C. Dale, I.P. Touw, et al. 2017. Severe congenital neutropenias. Nat. Rev. Dis. Primers 3:17032.
3. Hammond, W.P., T.H. Price, L.M. Souza & D.C. Dale. 1989. Treatment of cyclic neutropenia with granulocyte colony-stimulating factor. N. Engl. J. Med. 320:1306–1311.
4. Dale, D.C. & K. Welte. 2011. Cyclic and chronic neutropenia. Cancer Treat. Res. 157:97–108.
5. Newburger, P.E., T.N. Pindyck, Z. Zhu, et al. 2010. Cyclic neutropenia and severe congenital neutropenia in patients with a shared ELANE mutation and parental haplotype: evidence for phenotype determination by modifying genes. Pediatr. Blood Cancer 55:314–317.
6. Dale, D.C., A. Bolyard, T. Marrero, et al. 2017. Long-term effects of G-CSF therapy in cyclic neutropenia. N. Engl. J. Med. 377:2290–2292.
7. Makaryan, V., C. Zeidler, A.A. Bolyard, et al. 2015. The diversity of mutations and clinical outcomes for ELANE-associated neutropenia. Curr. Opin. Hematol. 22:3–11.
8. Klimiankou, M., S. Mellor-Heineke, O. Klimenko, et al. 2016. Two cases of cyclic neutropenia with acquired CSF3R mutations, with 1 developing AML. Blood 127:2638–2641.
9. Lei, J. & M.C. Mackey. 2011. Multistability in an age-structured model of hematopoiesis: cyclic neutropenia. J. Theor. Biol. 270:143–153.
10. Lei, J. & M.C. Mackey. 2014. Understanding and treating cytopenia through mathematical modeling. Adv. Exp. Med. Biol. 844:279–302.
11. Freund, M.R., S. Luft, C. Schober, et al. 1990. Differential effect of GM-CSF and G-CSF in cyclic neutropenia. Lancet 336:313.
12. Welte, K., C. Zeidler, A. Reiter, et al. 1990. Differential effects of granulocyte-macrophage colony-stimulating factor and granulocyte-stimulating factor in children with severe congenital neutropenia. Blood 75:1056–1063.
13. Krance, R.A., W.E. Spruce, S.J. Forman, et al. 1982. Human cyclic neutropenia transferred by allogeneic bone marrow grafting. Blood 60:1263–1266.
14. Kollner, I., B. Sodeik, S. Schreek, et al. 2006. Mutations in neutrophil elastase causing congenital neutropenia lead to cytoplasmic protein accumulation and induction of the unfolded protein response. Blood 108:493–500.
15. Grenda, D.S., M. Murakami, J. Ghatak, et al. 2007. Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis. Blood 110:4179–4187.
16. Nanua, S., M. Murakami, J. Xia, et al. 2011. Activation of the unfolded protein response is associated with impaired granulopoiesis in transgenic mice expressing mutant Elane. Blood 117:3539–3547.
17. Nustede, R., M. Klimiankou, O. Klimenko, et al. 2016. ELANE mutant-specific activation of different UPR pathways in congenital neutropenia. Br. J. Haematol. 172:219–227.
18. Korkmaz, B., M.S. Horwitz, D.E. Jenne & F. Gauthier. 2010. Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacol. Rev. 62:726–759.
19. Benson, K.F., F.Q. Li, R.E. Person, et al. 2003. Mutations associated with neutropenia in dogs and humans disrupt intracellular transport of neutrophil elastase. Nat. Genet. 35:90–96.
20. Tidwell, T., J. Wechsler, R.C. Nayak, et al. 2014. Neutropenia-associated ELANE mutations disrupting translation initiation produce novel neutrophil elastase isoforms. Blood 123:562–569.
21. van Galen, P., A. Kreso, N. Mbong, et al. 2014. The unfolded protein response governs integrity of the haematopoietic stem-cell pool during stress. Nature 510:268–272.
22. Leale, M. 1910. Recurrent furunculosis in infant showing unusual blood picture. JAMA 54:1845.
23. Souza, L.M., T.C. Boone, J. Gabrilove, et al. 1986. Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. Science 232:61–65.
24. Welte, K., J. Gabrilove, M.H. Bronchud, et al. 1996. Filgrastim (r-metHuG-CSF): the first 10 years. Blood 88:1907–1929.
25. Heijmans, J., J.F. van Lidth de Jeude, B.K. Koo, et al. 2013. ER stress causes rapid loss of intestinal epithelial stemness through activation of the unfolded protein response. Cell Rep. 3:1128–1139.
26. Clevers, H. 2013. The intestinal crypt, a prototype stem cell compartment. Cell 154:274–284.
27. Foudi, A., K. Hochedlinger, D. Van Buren, et al. 2009. Analysis of histone 2B-GFP retention reveals slowly cycling hematopoietic stem cells. Nat. Biotechnol. 27: 84–90.
28. Wilson, A., E. Laurenti, G. Oser, et al. 2008. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 135: 1118–1129.
29. Hsu, Y.C., H.A. Pasolli & E. Fuchs. 2011. Dynamics between stem cells, niche, and progeny in the hair follicle. Cell 144: 92–105.
30. Tian, H., B. Biehs, S. Warming, et al. 2011. A reserve stem cell population in small intestine renders Lgr5-positive cells dispensable. Nature 478: 255–259.
31. Zeidler, C., L. Boxer, D.C. Dale, et al. 2000. Management of Kostmann syndrome in the G-CSF era. Br. J. Haematol. 109: 490–495.
32. Liu, L., M. Zhao, X. Jin, et al. 2019. Adaptive endoplasmic reticulum stress signalling via IRE1α-XBP1 preserves self-renewal of hematopoietic and pre-leukaemic stem cells. Nat. Cell Biol. 21: 328–337.