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Congenital Neutropenia – Section 5

EHA Educational Lecture, Session Congenital Neutropenia: Severe Congenital Neutropenia-Biological Insights

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Take-home messages:

- Severe congenital neutropenia (SCN) is a genetically heterogeneous condition, with autosomal dominant, recessive, and sporadic forms. The underlying biology of how these mutations cause severe neutropenia is diverse and still only partly understood.
- Leukemia predisposition is a major concern in different genetic subtypes of SCN. Somatic mutations in the genes encoding the G-CSF receptor (*CSF3R*) and the transcription factor *RUNX1* are strongly associated with, but not sufficient for, progression of SCN to myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Additional events involving alterations of epigenetic regulators in hematopoietic stem and progenitor cells are needed for malignant transformation of SCN.
- The sequence of appearance of mutations in *CSF3R* (early in neutropenic phase) and *RUNX1* (shortly before overt MDS or AML) suggests that aberrant signaling from truncated CSF3R and pro-inflammatory reactions facilitate malignant transformation driven by *RUNX1* mutations.

Introduction

Severe congenital neutropenia (SCN) is a genetically heterogeneous condition usually diagnosed in early childhood.[*1] Autosomal dominant mutations in *ELANE*, the gene encoding neutrophil elastase, are the most frequent cause of SCN and are found in approximately 45% of patients.[*2,3,4] Deleterious mutations in *HAX1*, encoding a protein involved in mitochondrial integrity and cytoskeleton organization, cause a recessive form of SCN confined to consanguineous populations, representing less than 5% of SCN patients.[*5] Other genetic subtypes of SCN are even more rare and in a significant proportion of cases (approximately one-third) an underlying genetic defect has not yet been identified.[*1] Most often, bone marrow aspirates from SCN patients show a maturation arrest at the promyelocyte stage, but how the variety of mutations cause this arrest and the resulting severe defect in neutrophil production is largely unclear. Treatment with colony stimulating factor 3 (CSF3), in clinical practice better known as granulocyte colony-stimulating factor (G-CSF), neupogen or filgrastim, alleviates the neutropenia in most SCN patients. A major concern in treating SCN patients with G-CSF is the highly increased risk of developing myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). Progression to MDS/AML is associated with the appearance of hematopoietic clones with somatic mutations in the gene encoding the G-CSF receptor (*CSF3R*), resulting in a truncated form of CSF3R with defective internalization and aberrant signaling properties.[*6,*7,8] These clones can persist for months or years before MDS/AML, most frequently characterized by additional mutations in *RUNX1*, becomes clinically overt.[*6,9] This educational lecture addresses the recent efforts to obtain insights into how SCN develops and predisposes to MDS/AML and how this knowledge can be exploited for designing preemptive strategies to avoid malignant transformation.

Current state-of-the-art

Before the introduction of G-CSF therapy in the early 1990s, SCN patients regularly succumbed to the life-threatening bacterial infections, to which they lacked an adequate host-defense. Because more than 90% of SCN patients respond well to G-CSF treatment, this severe complication improved dramatically.[10] Intriguingly, most patients respond to G-CSF therapy despite the fact that endogenous G-CSF serum levels are normal or even slightly elevated, raising the question why administration of exogenous G-CSF is effective. A plausible explanation for this apparent paradox is that SCN patients are hampered in their basal state of granulopoiesis, driven by the transcriptional regulator C/EBPα.[11] However, at high dosages of G-CSF a state termed "emergency" granulopoiesis is induced, which is controlled by C/EBPβ. In healthy individuals, emergency granulopoiesis is activated during phases of bacterial infections. In SCN patients, sustained G-CSF treatment activates C/EBPβ-dependent emergency granulopoiesis, alleviating the severe neutropenia in the absence of infections.[11]

An intriguing, yet puzzling characteristic of SCN is its wide genetic diversity. Currently, mutations in more than 20 genes have

The authors have no conflicts of interest to disclose.

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HemaSphere (2020) 4:S2
Received: 27 January 2020 / Accepted: 27 March 2020

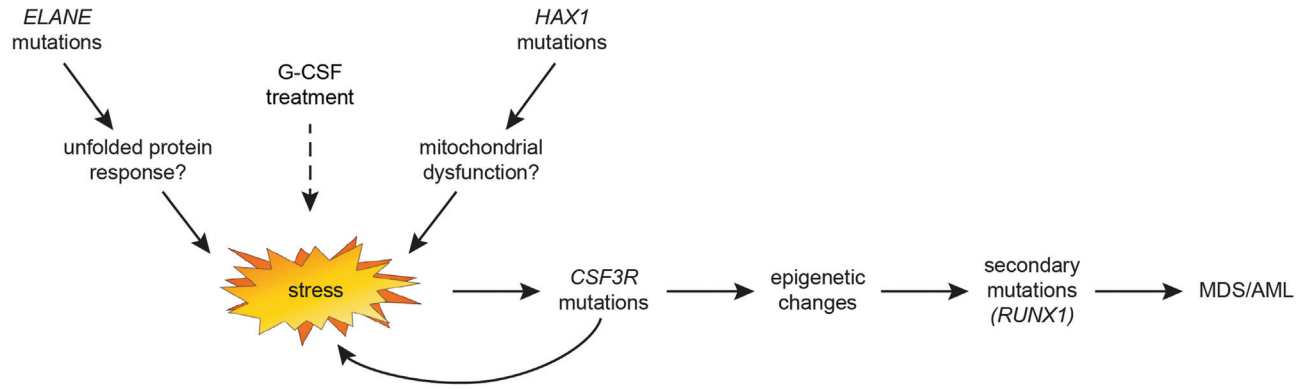

Figure 1. Simplified scheme of the sequential steps leading to malignant transformation of SCN.

been implicated as the cause of SCN.[*1] These mutations affect the function of a variety of proteins that exert widely diverse intracellular functions, among which protein trafficking, actin cytoskeleton organization, mitochondrial integrity, transcriptional control and signal transduction. The most common SCN-causative mutations, that is, those in *ELANE*, are distributed over the entire exome and more than 100 different mutations have been identified.[*1,3,4] This poses yet another conundrum: How do these *ELANE* mutations, all considered to be gain of function mutations, result in severe neutropenia? A prevailing hypothesis is that *ELANE* mutations cause misfolding of the mutant neutrophil elastase (NE) protein, evoking an unfolded protein response (UPR) and cellular stress, eventually resulting in accelerated cell death beyond the promyelocyte stage.[12] Because not all *ELANE* mutations predictably result in protein misfolding, alternative hypotheses as to how they may cause neutropenia have been proposed, for example, involving altered substrate specificities and/or mistrafficking of mutant NE from their normal residence in granules.[13] Further adding to the complexity, *ELANE* mutations similar to those found in SCN can also cause cyclic neutropenia (CyN), a related but clearly distinct form of neutropenia.[13] Although CyN patients are also treated with G-CSF during the cyclic phases of nadir, expansion of *CSF3R* mutant clones and progression to MDS/AML are exceptional events. Efforts to address this in mouse models are hampered by the fact that many SCN-causing mutations introduced in mice did not lead to a neutropenic state comparable to that seen in patients.[14] For some genetic subtypes of SCN, zebrafish models may be a valuable alternative for *in vivo* studies, while patient-derived induced pluripotent cell lines (iPSCs) could be a valuable alternative for in vitro studies.[14,15]

Mouse models have also been used to study the cooperation of patient-derived mutations in *CSF3R* and *RUNX1* in leukemic progression and the role of sustained G-CSF treatment herein. These mice developed a G-CSF-driven preleukemic syndrome with excess blasts in the peripheral blood, but did not develop overt AML. Serial transplantation of these preleukemic cells in secondary and tertiary recipients resulted in AML, which was independent of G-CSF but driven by a novel mutation affecting TET2 levels. This was associated with strongly elevated inflammatory responses within the hematopoietic stem and progenitor cell (HSPC) population. These data indicate that under sustained G-CSF treatment, defects driving inflammatory processes are critical for full malignant transformation of SCN in conjunction with *CSF3R* and *RUNX1* mutations.[16]

To address why SCN patients have a predisposition for developing MDS or AML, patient-derived iPSCs are useful models in which the underlying molecular, biochemical and cellular mechanisms can be dissected. Such experiments, directed towards the consequences of *CSF3R* and *RUNX1* mutations in the SCN versus control backgrounds are in progress. In addition, mechanisms of oxidative and inflammatory stress responses as potential drivers of malignant transformation can be conveniently studied in these models. A simplified scheme of how these sequential steps in malignant transformation might take place is shown in Fig. 1.

Outlook

Despite our increasing knowledge of the genetic defects underlying SCN, many issues concerning how these mutations cause neutropenia and predispose to MDS or AML remain to be resolved. Patient-derived iPSC models may help to gain further insight into these mechanisms. Furthermore, they can be used to develop new treatment modalities, particularly for patients who fail to respond to G-CSF therapy.

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