Review Article

Recent Advances of Therapeutic Strategies Based on the Molecular Signature in Non-Syndromic Congenital Neutropenia

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Abstract

Neutropenia has been defined to be classified into transient and chronic forms based upon duration and acquired or congenital forms. Chronic neutropenia is considered as congenital, cyclic, autoimmune and idiopathic categorizes, where congenital and cyclic categories are hereditary forms of neutropenia, but the idiopathic and autoimmune are known as acquired neutropenia. In the present review, we focus highly on the genetic subtypes of non-syndromic congenital neutropenia and therapeutic strategies regarding to recent evidence. All aspects of molecular pathogenesis and biology have not been clarified so far; therefore, further in-depth understanding of the molecular mechanisms is needed to reveal roles of molecular mechanisms, and pathways involved in the development of congenital neutropenia and to provide a basis for achieving novel therapeutic strategies.

Keywords: *Neutropenia, Molecular mechanisms, Therapeutic strategies, Mutations*

Background

Definition of Neutropenia

Neutropenia is considered as a blood neutrophil count $<1{\cdot}5 \times 10^9$/l, that is commonly detected in 6–8% of all newborns during their neonatal intensive care unit (NICU) stay [1]. Neutropenia is considered as a reduction in the number of circulating neutrophils. The presence of neutropenia can be associated with an underlying systemic or hematological disease. Neutropenia is labelled as mild, moderate- and severe neutropenia [2-4]

In an asymptomatic neutropenic neonate, the association between low ANC and the risk of infection will be considered speculative, and children's neutropenia can be involved in Kostmann syndrome or chemotherapy. It has been defined that an ANC <500/μL may be potentially associated with an increased risk of secondary infections, especially when neutropenia continues for more than a few days [5, 6]

A number of factors have been defined to be capable of influencing the susceptibility of pyogenic bacteria, enteric bacteria, and some fungi in patients suffering from neutropenia including severity of neutropenia, bone marrow myeloid reserves, speed of onset and duration of the neutropenia, absolute monocyte count (AMC), and

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the functional situation of phagocytes [7, 8]. Overall, many etiologies are involved in pediatric neutropenia [7, 8]. The duration of neutropenia is of particular importance and there is needed for further assessment of neonates suffering from mild-moderate neutropenia for >7–10 days [6, 9]

Moreover, the risk of life threatening bacterial can be markedly increased, when the neutrophil count is less than 1.0×10^9/L [2-4]. The appropriate reference range plays a key role in preventing the mistake of detecting a child with neutropenia. The appropriate reference range plays a key role in preventing the mistake of detecting a child with neutropenia. Based on the available date, Caucasian white people show a higher neutrophil count compared to African, Arabic-Middle Eastern origin and Afro-Caribbean. It has been shown that 25% of African people have shown neutrophil counts of less than 1.0× 10 / liter, where this condition called "ethnic neutropenia" and exhibiting little clinical consequence [10]. It has been indicated that neutropenia is capable of affecting up to 50% of very low birth weight infants as compared term newborns, leading to an increased risk of sepsis and mortality, as well as an enhanced risk of infection secondary to neutropenia [9,11,12]

It is noteworthy that ethnic neutropenia has been found to be persistently seen until adulthood. Certain types of neutropenia (e.g., benign neutropenia of childhood) can present with profound neutropenia yet have little clinical consequence. Some types of neutropenia (i.e., benign neutropenia of infancy/ childhood) can be associated with profound neutropenia (ANC below 100/uL), but exhibit few clinical outcomes [13]. On the other hand, 6% of premature or small for gestational age newborns are likely to exhibit ANC lower than 1·0 × 109/l [14,15]

Neutropenia is classified into acute (or transient) and chronic forms based upon duration and acquired or congenital form [16]. Chronic neutropenia is considered as congenital, cyclic, autoimmune and idiopathic categorizes, where congenital and cyclic categories are hereditary neutropenia, but the idiopathic and autoimmune are known as acquired neutropenia [8,17]

A heterogeneous group of diseases can potentially lead to the occurrence of neutropenia such as benign transient post-viral suppression, and overwhelming systemic disease and the clinical pattern of causes and outcome are different at different ages for children and adults [10]. Neutropenia arises as an adverse reaction with certain drugs or chemotherapy. It is important to note that recognizing the causes of neutropenia may be difficult; therefore, the cause of the illness plays a key role in its management and prognosis.. It is noteworthy that the mechanisms of isoimmune vs alloimmune are involved in the onset of neutropenia at birth and for the first few months, where the risk of infection with severe bacteria may increase [15]. Neutropenia in children may be diagnosed accidentally when minor symptoms are recognized, but some issues are of particular importance, including the discrimination of acute or benign causes and severe congenital neutropenia as a rare condition, as well as neutropenia linked to hematological disorder [10]

Hereditary neutropenia (congenital neutropenia)

Congenital neutropenia is considered to be a rare primary immunodeficiency disease and is characterized by chronic neutropenia linked to genetically heterogeneous phenotypic traits. It occurs in the first year of life as a recurrent fever, which usually depends on the type of infection. However, it should be noted that there are difficulties in identifying idiopathic, autoimmune or congenital neutropenic conditions [15]. Therefore, the genotype plays a key role in distinguishing neutropenic types, but cannot be easily applied during primary evaluation [18]. However, it is important to note that severe and recurrent infections are commonly seen in congenital disorders [19]. Furthermore, some parts of an organ involved in the diseases may not be visible in the initial stage [18]. It has been documented that different distinctive syndromes are involved in congenital neutropenia in children. Family history, physical examination, and laboratory testing may lead the physician to a conformational genetic test [20]. Importantly, the genetic diagnosis has been described as an important criterion for categorizing patients. It has been proven that mutations in ELANE, HAX1, GFI1, or WAS are related to non-syndromic variants of congenital neutropenia. On the other hand, congenital neutropenia syndromes are involved in different gene defects and mutations including encoding genes of mitochondrial proteins (Gene defects: AK2, TAZ), encoding genes of ribosomal proteins (Gene defects: Shwachman-Bodian-Diamond syndrome (SBDS), RMRP (RNA component

of mitochondrial RNA processing endoribonuclease)), and controlling genes of glucose metabolism (Mutations: SLC37A4 [G6PT1], G6PC3) or controlling genes of lysosomal function (Mutations: LYST (Lysosomal Trafficking Regulator), AP3B1, RAB27A, VPS13B, ROBLD3/p14), [20,21]. Therefore, we have described the genetic cause involved in the non-syndromic congenital neutropenia that is characterized by maturation or deficiency.

Non-Syndromic Congenital Neutropenia

Severe congenital neutropenia (SCN) and cyclic neutropenia (CYN)

Multiple hereditary syndromes play a key role in the development of SCN, for which there is strong evidence, and the similar role has been defined for the underlying genetic defects, however, its etiology is unknown in many patients [22,23]

Congenital neutropenia is known as a rare disease, which its prevalence ranged from 3 to 8.5 cases per million [24]. The autosomal dominant disorders are widespread throughout the world, while an increasing body of evidence suggests that recessive disorders are commonly diagnosed in consanguineous populations. It is noteworthy that recurrent bacterial infections are predominantly appeared in mucous membranes, skin and oral cavity of patients suffering from SCN and disease accompanied with aphthous stomatitis, periodontitis, and abscesses and damaged teeth associated with frequent gingivitis [25,26]

HAX1 mutations-related SCN has been estimated to be high due to due to consanguineous marriages of Turkish and Arabic families. Confirmation of the diagnosis of SCN by determining the ELANE mutation should be first taken into consideration unless other evidence is achieved including family history and clinical clues obtained from the physical assessment and laboratory findings. Regarding the hereditary factors, it has been revealed that Caucasian white people show a higher neutrophil count compared to African, Arabic-Middle Eastern origin and Afro-Caribbean. In comparison, African heritage a lower neutrophil count, when compared with people white European or Asian origin. Moreover, polymorphism in the Duffy antigen/ chemokine receptor has been indicated as a key factor associated with neutropenia in African heritage [27, 28]. Overall, mutations and deficiency in many genes have been revealed to be involved in SCN. These include the ELA2, HAX1, GFI1, WASP, VPS45, JAGN, SLC37A4, G6PC3 and CSF3R. We focus highly on the genetic subtypes of non-syndromic congenital neutropenia that are characterized by maturation or deficiency.

Autosomal Dominant

CYN has been defined as rear and autosomal dominantly inherited disorder that is characterized by oscillations in neutrophils and recurrent episodes of severe neutropenia with an average 21-day interval. It can be clinically exhibited recurrent acute stomatologic disorders (e.g., mainly aphthae), fever, painful oral ulcers, recurrent oropharyngeal infections [25, 29]. ELANE□CYN is attributable to an increased risk of serious infections, especially for children [18]. Genetic findings have attributed a role to mutation of neutrophil elastase gene, ELANE or ELA2 in developing CYN [30,31]. ELANE mutations are responsible for CYN and SCN, which are considered as bone marrow failure syndromes. The mutation has been estimated to be found in 80- 100% of subjects of CN and in 63-63% of SCN cases [32,33]. To best of our knowledge, more than 50 mutations have been described for of ELANE, where some of them are attributable to both SCN and CYN patients and some are responsible for SCN cases [34,35]. Emerging evidence has indicated that different functional perturbations in the ELANE protein are likely responsible for the maturation arrest in granulopoiesis [36]

Other study supporting the existence of unfolded protein response model for the pathogenesis of SCN linked to ELA2 mutations [35], the accumulation of misfolded proteins in the endoplasmic reticulum has been indicated to trigger the UPR pathway as a cellular stress response. The UPR pathway plays its role by triggering protein kinase RNA-like endoplasmic reticulum kinase (PERK), transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1). Activation of these key regulators reduces misfolded proteins via three mechanistically distinct arms including attenuated global protein synthesis, transcription of several ER-resident proteins (I.e., ER chaperone proteins) as well as via triggering ER-associated protein degradation pathway of misfolded proteins [37,38]. ATF6 has been shown to

participate in the regulation of master-chaperone (heat shock protein 5 (HSPA5), [39]

The fundamental question is how similar mutations can lead to distinct phenotypes, where are related to SCN and CYN. As a matter of fact, the same ELANE mutants can lead to the UPR in SCN patients, while not in patients suffering from CYN [39, 40]. Several mutations have been defined for SCN and CYN, where an increasing body of evidence suggests that there is no definite genotype-phenotype relationship [25,31]. A previous study indicated that SCN and CYN in patients with a shared ELANE mutation and paternal haplotype can play a role for determining congenital neutropenia phenotypes via modification of genes, and categorizing CYN and SCN in a spectrum of phenotypes, indicating the different degrees of the same disease processes [41]

Additionally, siblings sharing a similar mutation of ELANE can be capable of developing various phenotypes. Regarding tow aforementioned evidence, increasing evidence suggests that some scholars regard SCN and CYN as two sides of a coin [41, 42]

Growth factor independence-1 (GFI1) plays a key role in granulopoiesis in both mice and humans. Knockout (KO) murine GFI1 has been demonstrated to be crucial for neutrophil differentiation [43], which its knockout model showed myeloid differentiation arrest, leading to SCN [43, 44]. GFI1 has six C_2H_2-type zinc fingers and a SNAG domain that are involved in its repressor activity by binding DNA. The mutated protein loses the ability to bind to DNA, and consequently leads to stopping repressor activity on target genes.

This mutation has been found in patients who suffered from an inherited dominant bleeding disorder with anisopoikilocytosis and moderate congenital macrothrombocytopenia (CMTP), [45]

Whilst there is often experimental evidence that mice exhibited severe neutropenia and defects in the HSC fraction and in B cells and T helper cells due to a lack of truly Gfi1 [46-48]. GFI1mutantatin leads to elevated numbers of monocytes and decreased numbers of CD4-T and B cells. Overall, Gfi1 role in controlling the function of myeloid and lymphoid cells has been confirmed [48, 49]. Heterozygous mutations of GFI1 have been demonstrated in patinas suffering from SCN and CYN [50, 51], which SCN patients exhibit abnormal promyelocytes accumulate. Heterozygous human mutations in GFI1 are predominantly linked to the dominant negative p.N382S and p.K403R substitutions and a reduced level of neutrophil elastase result in GFI1 deficiency consequently has accompanied by additional defects of lymphocyte proliferation [45,52]. The GFI1N N382S mutation has been found to contribute to the inhibition of GFI1 DNA binding, leading to a dominant-negative block to murine granulopoiesis via colony stimulating factor-1 (CSF1), [52]

An increasing body of evidence indicates the interaction of NEDD4-binding protein 2-like 2 with neutrophil elastase and GFI1, and potentially leading to GFI1 mutations and decrease in ELANE expression in GFI1 deficiency [45,53]. It has been previously demonstrated that Gfi1 is capable of regulating the G-CSF signaling and development of neutrophil via the Ras activator Ras guanine-releasing protein 1 (RasGRP1), [54]

Autosomal recessive severe congenital neutropenia

Kostmann syndrome (HAX1-neutropenia)

Kostmann syndrome is defined as an autosomal recessive disorder, which is now known as SCN has been characterized by severe neutropenia and onset of severe bacterial infections.

As mentioned, patients who suffer from autosomal-dominant SCN exhibited the ELANE gene. Mutations arrest at the promyelocyte stage of neutrophil development has been described to be the result of this mutation and consequently result in ineffective neutrophil production, as well as cognitive impairments, delays, and epilepsy [55,56]. Biallelic mutations in the HAX1 gene have been proved to develop autosomal SCN. Maturation arrest' at the promyelocyte stage of neutrophil development is very similar to those seen in patients with mutations in ELANE.

It has been defined that deficiencies in HAX-1 expression are responsible to a genetic alteration contributing to the etiology and pathophysiology of certain clinical situations including SCN and/ or Kostmann syndrome (HAX1-neutropenia), and central nervous system or neurodevelopmental delays, as well

as abnormalities [57]

HAX1 deficiency, as other genetic subgroups of SCN, has been found to be capable of increasing the myeloid cell apoptosis [58-60]. It seems that there are other functions be associated with the cause of SCN; different HAX1 protein isoforms may be involved in its functions [61]. It is noteworthy that a more previous data has suggested a role for HAX-1 in the regulation of apoptosis, where apoptosis is capable of controlling neutrophil development [61]

Genotype-phenotype correlations have been identified (allelic heterogeneity) to be capable of affecting HAX1 transcript variants I, leading to neutropenia [31, 61, 62]. Additionally, the pathological mutation has been found to be removed from variant II, because of splicing out of the 5′end of exon 2 [61]

Accumulating evidence revealed that two phenotypes of HAX-1 deficiency are implicated in the presence of two isoforms of the HAX-1 protein. HAX-1 variant 001 and 004 (as mutation) are considered to be corresponding for the severe course of disease with SCN. Mutations in isoform 001 can lead to the development of the milder course of disease with isolated SCN because of splicing out of the 004 [57]. HAX1 mutations have been identified to be linked to the ethnic composition, where most subjects with Kostmann syndrome belonged to consanguineous marriages from Middle-Eastern descent [31, 63]

Future investigations can lead to an increased understanding of the complexity of multifunctional roles of HAX-1 in different cellular processes and human diseases (e.g., hematopoietic and malignancies disease, severe congenital neutropenia).

The iPSC differentiation system is suitable for the study of the clinical phenotype of Kostmann SCN and to develop new therapeutic options.

The patient-derived HAX1W44X-iPSC has been indicated to be capable of recapitulating the Kostmann syndrome phenotype in vitro, where an increasing body of evidence exhibited HAX1 mutations as a monogenic lesion of the disease. As a matter of fact, targeting the genetic correction of the HAX1 gene has also been considered as a means of repairing patient-derived iPSCs, where was capable to induce pluripotent stem cells. HAX1 and HCLS1 have been demonstrated to play their key roles in a large dysregulated genetic network and highlighted HAX1 involvement in other cellular signaling pathways [64]. A recent study demonstrated that HAX-1 play a fundamental role in the regulation of oxidative.

Stress and SERCA2a degradation, where it is involved, not only in calcium homeostasis, but also in the cell survival pathways and can play a key role in cardioprotection by regulating SERCA2a levels. A increasing body of evidence suggests that HAX-1 are not only responsible for the SR Ca2+ transport but also play an important role in multiple tissues and diseases, indicating the important nature of NOX4 derived ROS [65]

One of the questions that remain about the autosomal recessive of SCN is why the clinical manifestations in these patients are markedly restricted to the brain and the bone marrow [61], while ubiquitous expression of HAX1 has been revealed across various human tissues? [61, 62, 66]. An increasing body of evidence highlighted HAX-1 as a multifunctional protein. Aforementioned questions can be likely associated to different splice variants, by which isoforms may be encoded with several tissues' distribution and/or *many* functions and specific expression of the different HAX-1-interaction can potentially lead to subcellular localization and cellular function of HAX-1 in certain tissues [61]. Therefore, further in-depth understanding of the molecular mechanisms is needed to reveal roles of HAX-1 in various cellular processes and different tissues.

Conclusion

It should be taken into consideration that all aspects of molecular pathogenesis and molecular biology have not been clarified so far, decision making on treatment are currently sophisticated and limited to G-CSF and *HSCT*. Further in-depth understanding of the molecular mechanisms is needed to reveal roles of HAX-1 in various cellular processes and different tissues, where an increasing body of evidence highlighted HAX-1 as multifunctional protein, specific expression of the different HAX-1-interaction can potentially lead to subcellular localization and cellular function of HAX-1 in certain tissues. An increasing body of knowledge is not only relevant for individualized diagnostic and

therapeutic advice for congenital neutropenia, but also for the scientific community in general. Nevertheless, many questions remain unanswered,

A variety of diagnostic and therapeutic approaches to preventing non-syndromic congenital neutropenia are currently being achieved, however, all aspects of molecular pathogenesis and molecular biology have not been deeply understood, further advances are needed for providing both a better understanding of the involvement of molecular mechanisms, and pathways involved in the development of congenital neutropenia and a basis for providing novel therapeutic strategies.

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Conflict of Interest: Non

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