Comment on Tidwell et al, page 562

Severe congenital neutropenia: new lane for *ELANE*

Niels Borregaard[1] [1]UNIVERSITY OF COPENHAGEN

In this issue of *Blood*, Tidwell et al[1] demonstrate that mutations in the start codon (protein synthesis is initiated at the codon ATG) of neutrophil elastase (*ELANE*) result in the production of N-terminally truncated elastase, which mislocates to the nucleus and results in severe congenital neutropenia (SCN).

SCN is a rare condition with a serious impact on heath and quality of life. Although treatment with granulocyte colony-stimulating factor (G-CSF) largely restores circulating neutrophil counts and effectively reduces infections, the condition carries a risk of progression to acute myeloid leukemia of 2.3% per 10 years.[2]

A major breakthrough in understanding the genetic background of SCN and its more benign relative, cyclic neutropenia, was the discovery by Marshall Horwitz and David Dale and colleagues that virtually all forms of cyclic neutropenia, and most forms of autosomal-dominant SCN, which covers about 70% of the cases, are caused by mutations in the coding region of *ELANE*, the gene for neutrophil elastase,[3,4] one of the 4 serine proteases localized to azurophil granules of neutrophils along with the commonly used neutrophil marker, myeloperoxidase. These seminal papers were presented before the era of exome sequencing and were the result of meticulous studies of pedigrees of patients that made it possible to narrow down the gene defect to chromosome 19p13.3 and then verify that mutations were present in *ELANE*.

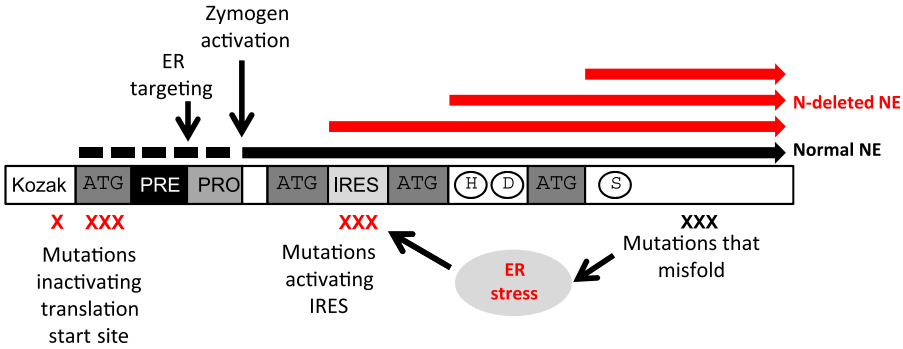
Why should mutations in the gene for elastase cause such problems? Although it is still not evident why *ELANE* mutations result in cyclic neutropenia, it is more easily comprehended that mutations in *ELANE*, one of the genes most abundantly expressed in promyelocytes,[5] may perturb the further development of these cells when transcription of such mutated *ELANE* is at its peak: but how? Several mechanisms have been offered. One explains elastase as a transmembrane protein with SCN mutations preventing its sorting to granules, resulting in routing to the plasma membrane, where it may cause havoc.[6] Another argues that mutations result in misfolding of elastase, which exceeds the capacity of the endoplasmic reticulum for corrections and induces an unfolded protein response leading to death of the cells,[7] much akin to the necrosis of liver cells in severe forms of α-1–antitrypsin deficiency.[8] Why G-CSF should ameliorate this is not quite evident, but it is perhaps the result of reduction in production time and hence the amount of protein synthesized at the promyelocyte stage before other transcription factors take over and shut down the production of the offending misfolded protein.

In this issue of *Blood*, a novel mechanism is put forward and supported by several lines of evidence.[1] The authors identified 8 cases of SCN where the mutations of *ELANE* affect 1 of the 3 nucleotides of the canonical translation initiation codon, ATG, which puts methionine on as the first amino acid when protein synthesis starts in all species. When this is mutated, the translational machinery will skip this site for initiation of translation and search for alternative ATG sites further downstream that satisfy the minimal requirements for initiation of translation, and such are indeed present in *ELANE* but alas result in shortened forms of elastase (see figure). Importantly, the part that is skipped codes for the signal peptide that guides proteins into the endoplasmic reticulum and eventually into granules. Instead, these N-terminally truncated forms are produced as cytosolic proteins. The presence of active proteases in the cytosol could certainly be expected to elicit apoptosis, but these truncated elastase forms are barely active and do not induce apoptosis. Instead, they stick to the nuclei of the cells. Although the authors demonstrate that such mutations negatively affect the proliferation of cells, it is still an open question whether the truncated forms by virtue of their mislocation and charge can block access of transcription factors to the nucleus necessary for further differentiation and hence explain the block of differentiation so characteristic of this condition.

These mutations are found only in a small minority of patients, but other *ELANE* mutations, located in the vicinity of the alternative translation initiations sites, make these more palatable for ribosomes to start translation at such internal ribosomal entry sites and putatively result in truncated elastase and SCN, even when the traditional start site is not mutated.

Although this paper concerns a small fraction of patients with a rare disorder, the study opens a new path for understanding how genetic defects affect cellular differentiation that may be relevant to other diseases, both congenital and acquired, not the least of which include the myelodysplastic syndromes and acute myeloid leukemia.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■



Mutations in the ATG site normally used as start of translation and other mutations, as indicated by the red X, result in the production of N-terminally truncated elastase lacking the signal peptide (red bars). These truncated proteins are liberated to the cytosol instead of being routed to the endoplasmic reticulum and granules as is wild-type elastase (black bar). See Figure 6 in the article by Tidwell et al that begins on page 562. ER, endoplasmic.

REFERENCES

1. Tidwell T, Wechsler J, Nayak RC, et al. Neutropenia-associated *ELANE* mutations disrupting translation initiation produce novel neutrophil elastase isoforms. *Blood*. 2013;123(4):562-569.

2. Rosenberg PS, Zeidler C, Bolyard AA, et al. Stable long-term risk of leukaemia in patients with severe congenital neutropenia maintained on G-CSF therapy. *Br J Haematol*. 2010;150(2):196-199.

3. Dale DC, Person RE, Bolyard AA, et al. Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia. *Blood*. 2000;96(7):2317-2322.

4. Horwitz M, Benson KF, Person RE, Aprikyan AG, Dale DC. Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. *Nat Genet*. 1999;23(4):433-436.

5. Theilgaard-Mönch K, Jacobsen LC, Borup R, et al. The transcriptional program of terminal granulocytic differentiation. *Blood*. 2005;105(4):1785-1796.

6. Benson KF, Li FQ, Person RE, et al. Mutations associated with neutropenia in dogs and humans disrupt intracellular transport of neutrophil elastase. *Nat Genet*. 2003;35(1):90-96.

7. Grenda DS, Murakami M, Ghatak J, et al. Mutations of the ELA2 gene found in patients with severe congenital neutropenia induce the unfolded protein response and cellular apoptosis. *Blood*. 2007;110(13):4179-4187.

8. Ordóñez A, Snapp EL, Tan L, Miranda E, Marciniak SJ, Lomas DA. Endoplasmic reticulum polymers impair luminal protein mobility and sensitize to cellular stress in alpha1-antitrypsin deficiency. *Hepatology*. 2013;57(5):2049-2060.

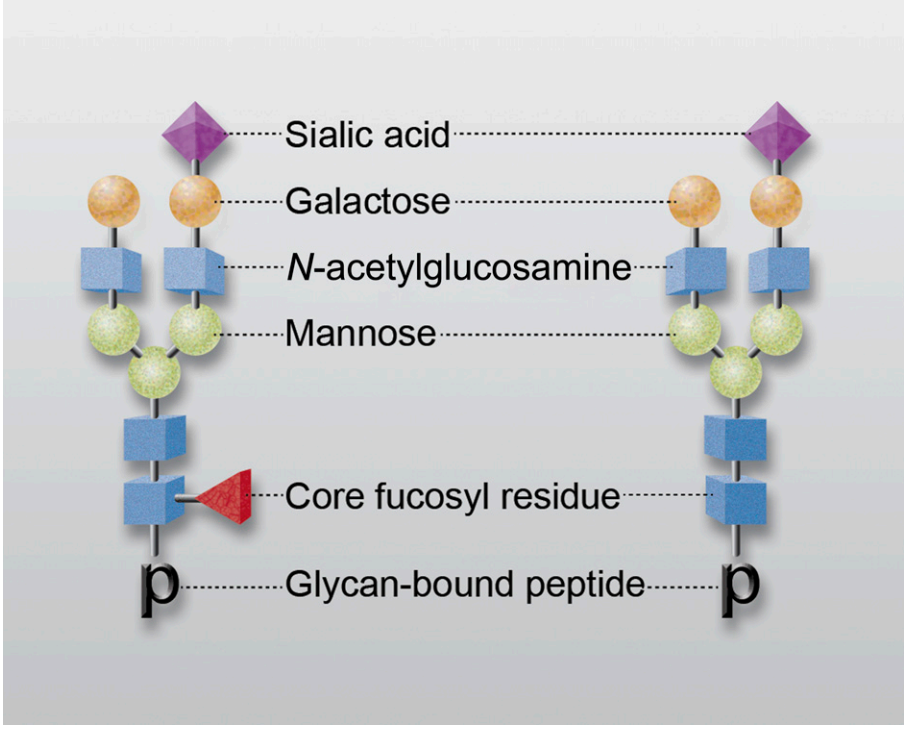
● ● ● PLATELETS & THROMBOPOIESIS

Comment on Kapur et al, page 471

Core fucosylation and IgG function in NAIT

Richard H. Aster[1,2] [1]BLOODCENTER OF WISCONSIN; [2]MEDICAL COLLEGE OF WISCONSIN

In this issue of *Blood*, Kapur et al show that maternal human platelet-specific antigen 1a (HPA 1a)-specific antibodies causing neonatal alloimmune thrombocytopenia (NAIT) possess oligosaccharides that are deficient in "core fucose" residues and appear to be more effective than fucosylated antibodies in promoting phagocytosis of antibody-coated platelets.[1]



Representative IgG-associated glycans with (left) and without (right) a core fucosyl residue (red triangle). Other saccharides are *N*-acetylglucosamine (blue), mannose (green), galactose (orange), and sialic acid (purple). "**p**" designates glycan-bound peptide in tryptic digest subjected to mass spectroscopic analysis. Professional illustration by Alice Y. Chen.

Each immunoglobulin G (IgG) molecule contains 2 oligosaccharide groups linked to asparagine residues at the 297 positions of the Fc domain. Each glycan usually consists of a complex heptasaccharide core containing *N*-acetylglucosamine (GlcNAc) and mannose to which variable numbers of galactose, fucose, sialic acid, and sometimes bisecting GlcNAc residues are attached (see figure). It is now well established that the character of these glycans can critically influence immunoglobulin function, particularly by modulating affinity for Fcγ receptors.[2-4] One of these posttranslational modifications, the addition of a fucose residue in α1,6 linkage to the first GlcNAc of the oligosaccharide core ("core fucosylation"), modulates the affinity of IgG Fc for the FcγRIII receptor expressed on natural killer cells, macrophages, neutrophils, and other cells. IgG molecules lacking a core-fucose residue bind more tightly to FcγRIII and exhibit enhanced cellular immune function, for example, are more effective in antibody-dependent cellular cytotoxicity.[5-7] The molecular basis for this effect was recently characterized by Ferrara and coworkers[8]; the potential advantage of using monoclonal antibodies lacking a core-fucose residue in cancer chemotherapy is currently under investigation.[3,9] Up to 30% of IgG molecules in normal human serum lack a core-fucose residue, but how core fucosylation is regulated, and the extent to which it influences the severity of antibody-mediated human disease, are poorly understood.

NAIT, a significant cause of morbidity and mortality in newborns, is caused by maternal antibodies specific for an HPA inherited by the fetus from its father.[10] The antigen against which these antibodies are most often directed is designated "HPA-1a." In a woman sensitized to HPA-1a and carrying a fetus at risk for NAIT, a tool capable of predicting NAIT severity could be extremely helpful in optimizing prenatal and perinatal management. Various studies have shown that serologic measurement of antibody potency alone is not sufficient for this purpose.[10]

In this issue of *Blood*, Kapur et al describe studies in which HPA-1a antibodies were isolated from serum of 48 women sensitized to HPA-1a who gave birth to an infant with NAIT.[1] The isolated immunoglobulins were digested with trypsin and subjected to nano liquid-chromatography tandem mass