sustaining the need to move rapidly to trials testing combination therapies. Another concern is the relatively high incidence of cutaneous malignancies in patients treated with BRAF V600E inhibitors along with other cutaneous adverse effects. The report of a patient on both BRAF and MEK inhibitors who developed a RAS-mutated pancreatic cancer deepens the need to study novel treatment approaches on carefully monitored clinical trials. 

There also remains the intriguing question of why some patients with BRAF V600E somatic, activating mutations of a hematopoietic lineage develop ECD vs LCH vs hairy cell leukemia. Is the cell context or maturational stage key to this question? And if the mutations arise in a similar precursor, what other factors direct the aberrant cell along such different neoplastic paths? Clearly, more work will be necessary and that work will require standardized diagnostic and response criteria. Such goals will also need to be applied to other histiocytic disorders, such as juvenile xanthogranulomatous disease and Rosai-Dorfman disease.

High aerial acts without a net are dangerous, but attract audiences. Bringing more attention to rare diseases like ECD will hopefully serve a similar purpose.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

REFERENCES


© 2014 by The American Society of Hematology

Erythroid mRNA processing: a “splice of life”

Don M. Wojchowski MAINE MEDICAL CENTER RESEARCH INSTITUTE

In this issue of Blood, Cheng and colleagues report first on intriguingly complex pre–messenger RNA (pre-mRNA) global alternate splicing (AS) events that occur in primary erythroblasts. Predominant regulatory roles then are implicated for a Muscleblind-like (MBNL) splicing factor, Mbnl1 (an AS regulator in pluripotent embryonic stem [ES] cells and myoblasts), and for nuclear distribution element like-1 (Ndel1) as a Mbnl1 target. Via loss-of-function (LOF) analyses, Mbnl1 and Ndel1 further are evidenced to support erythroblast development.

Editing of primary gene transcripts to yield variant exon–included vs. –excluded mRNA forms is an important mechanism for generating protein isoforms with diversified activities. To illustrate, one established hematologic example of such AS is FAS in which exon–6 inclusion yields a membrane–anchored proapoptotic ligand, whereas exon–6 excision generates soluble apoptosis antigen–1 with prosurvival properties. In higher eukaryotes, the majority of transcripts may, in fact, be subject to AS, a process which is regulated by one of the most sophisticated subcellular machines, the spliceosome (snRNPs U1-U6, plus ~200 polypeptides). In leukemogenic contexts, heightened attention to the spliceosome recently has arisen from associated newly discovered mutations, especially a SF3B1 component. Specifically, SF3B1 is mutated at substantially heightened frequencies in both myelodysplastic syndrome and chronic lymphocytic leukemia. Although more work is needed to establish functional significance, SF3B1’s knockdown in hematopoietic progenitors also generates ring sideroblasts.

Preclinical investigations also are providing new insight into selective regulators of AS and functional consequences. Here, major advances have been provided by the advent of approaches such as RNAseq and CLIP-seq as used to define RNA AS cis motifs together with factors acting at exon–intron borders. For erythropoiesis, global analyses of AS have been lacking to date. The present studies by Cheng et al use a murine fetal liver erythroid progenitor cell model first to identify ~600 AS events for transcripts with diverse gene ontologies. Beyond this, via analyses of pantemeric regions adjacent to splice sites (and comparisons to published CLIP-seq binding clusters), enrichment among erythroid AS events is defined for clusters predicted to bind MBNL splicing factors (Mbnl 1–3). Muscleblind is an AS factor that is involved in selective programs of splicing decisions (and was discovered in Drosophila as a regulator of muscle) and photoreceptor differentiation. In human myotonic dystrophies (DMs), MBNLs associate with abnormal 3’ CTP repeats in nuclear pre-mRNAs encoding DM protein kinase, a component of a DM toxic RNA model. Mice harboring Mbnl1–/– plus Mbnl2–/– alleles also recently have been shown to exhibit cardinal features of DM muscle disease. In additional contexts, extended roles of MBNLs are illustrated by regulation of FOXp1 AS in a context of ES cell pluripotency, and in insulin receptor transcript AS. In studies by Cheng et al, Mbnl1 was expressed in fetal liver erythroblasts, and its knockdown led to
increased blast cell size, attenuated hemoglobinization, and decreased frequencies of enucleated erythrocytes, indicative of hindered differentiation in the absence of Mbnl1. Upon ectopic expression of Mbnl1 (exon inclusion form), this LOF phenotype was partially reversed.

To initially seek Mbnl1 targets, gene expression patterns and published CLIP-seq analyses were analyzed, with Ndell pre-RNA as one confirmed target that also bound Mbnl1 in RNA immunoprecipitation and splicing reporter experiments. Ndell is a conserved coiled-coil protein (and apparent thiol-activated peptidase) with intriguing binding partners. These include LIs1 as mutated in lissencephaly, and DISC1 as a high-risk factor for schizophrenia. In proerythroblasts, Cheng et al go on to show that Ndell knockdown inhibits the formation of enucleated red cells, and that a 3’ exon inclusion form (but not exclusion form) partially rescues this knockdown phenotype. Overall, work by Cheng et al provides novel insight into global AS events during erythropoiesis, and specifically so in murine fetal liver erythropoiesis as a robust model system. Selective utilization of splicing regulators that target specific sets of AS events also is implicated, with Mbnl1 and Ndell as initially defined players. Among Mbnl1-3, additional roles for Mbnl2 in adult bone marrow erythropoiesis will be of interest to define in future studies. For Ndell, possible effects on erythroid blast growth, cytoskeletal features, and/or differentiation also merit extended attention. In a broader scope, RNAseq analyses also have been recently reported for developing human (pro)erythroblasts, and such contributions should enable further insight into AS regulation, specificity, and function during normal and dysregulated human erythropoiesis.

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

REFERENCES

© 2014 by The American Society of Hematology

THROMBOSIS & HEMOSTASIS

Comment on Proulle et al, page 611

Platelets as pivot in the antiphospholipid syndrome

Philip G. de Groot UTRECHT UNIVERSITY MEDICAL CENTER

In this issue of Blood, Proulle et al demonstrate that platelets are essential for the prothrombotic effects of anti–β2-glycoprotein I autoantibodies in a mouse model of the antiphospholipid syndrome (APS). APS is characterized by the presence of antiphospholipid antibodies in patients with thrombosis or pregnancy morbidity. Although its name suggests otherwise, antiphospholipid antibodies are not directed against phospholipids but toward plasma proteins with affinity for anionic phospholipids. Experiments in a mouse model for APS have shown that autoantibodies against the plasma protein β2-glycoprotein I are responsible for the increased risk of thrombosis. A complication in our understanding of the syndrome is that no physiological function has been assigned to β2-glycoprotein I and that men and mice without β2-glycoprotein I seem to be healthy. How antibodies directed against β2-glycoprotein I can lead to an increased risk of thrombotic complications is unsolved and the cause of vividious debates. A consensus now is that the autoantibodies induce a new activity for the protein. It has been shown that β2-glycoprotein I antibody complexes, but not β2-glycoprotein I alone, can activate different cell types that are involved in the regulation of hemostatic response, resulting in a prothrombotic state. A major question that remains is: “What are the target cells in vivo for the antibody–β2-glycoprotein I complexes?”

β2-glycoprotein I can exist in 2 completely different conformations. In plasma, it is present as a circular protein in which its N-terminal domain interacts with its C-terminal domain. After interaction with antibodies, the protein opens up and forms a hockey stick–like conformation. This stretched conformation now expresses a hidden epitope in its C-terminal domain that is involved in the binding of the antibody–β2-glycoprotein I complex to cells. To complicate matters, this complex is very sticky and binds in vitro to many different proteins and cellular receptors, questioning the value of the results of these experiments. Animal experiments are an essential tool to understand the consequences of cellular interactions of the autoantibodies–β2-glycoprotein I complexes. However, mouse models of APS have shown that endothelial cells, monocytes, and platelets all became activated when anti–β2-glycoprotein I antibodies were infused. The question remains whether 1 specific cell is the prime target or that the complexes can activate different cell types independently of each other. The experiments of Proulle et al showed that inhibition of platelet activation prevents the activation of endothelial cells. Thus platelets are the first target for the complexes, and products released from activated platelets are responsible for the local activation of endothelial cells.
Erythroid mRNA processing: a "splice of life"

Don M. Wojchowski