Ribosomal proteins in the news

This month's Journal Club will focus on a manuscript recently published in the prestigious journal, *Nature Genetics*. The manuscript is entitled, "Exome sequencing identifies mutations in *CNOT3* and ribosomal genes *RPL5* and *RPL10* in T-cell acute lymphoblastic leukemia" (De Keersmaecker et al, 2013). Since this manuscript deals with a topic which could have significant clinical implications for the DBA community, I thought it would be useful to recruit a physician as co-author this month and Dr. Jeffrey Lipton has graciously agreed to join me and his thoughts have been incorporated below.

The manuscript under consideration follows recent trends in using whole exome sequencing to identify the genes underlying human disease states. This same technology was, and is, being used to identify new DBA genes where the most notable success was in identifying the *GATA1* gene as the pathogenic lesion in certain DBA families. Here the disease investigated was T-cell acute lymphoblastic leukemia (T-ALL). This is not the first time that whole exome sequencing has been used to study human cancers. In fact, whole exome sequencing of cancers has been all the rage in recent years and the reason for this is the enormous wealth of information gained from these studies. Cancer cells are typically genetically unstable, so there are often many mutational changes in an advanced tumor. This makes sorting out driver mutations (those critical in the transformation of a normal cell to a tumor cell) from passenger mutations (those that contribute little or nothing to tumorigenesis but are just along for the ride) difficult to sort out. Some cancers, for example, chronic myelogenous leukemia have a single well-defined gene that drives tumor cell growth and are involved in most forms of this disease. Because of this well-defined driver gene it has been possible to develop drugs that target its gene product, making it the poster child for a remarkably effective therapy for this deadly disease (Wong & Mirshahidi, 2011). In contrast, other cancers, including T-cell acute lymphoblastic leukemia are far more heterogeneous and can be caused by mutations in a wider spectrum of genes. Because of this heterogeneity there is unlikely to be a one size fits all therapy for these types of cancers. Instead, by sequencing individual tumor genomes and identifying the different players involved in one tumor relative to another, physicians can take a more personalized approach to therapy specifically directed towards the drivers for a particular tumor. Thus, there is intense interest these days in using whole exome sequencing on a tumor by tumor basis to identify such drivers for both prognostic and therapeutic decisions.

This whole exome approach can occasionally identify issues relevant to DBA, as is the case in the manuscript under consideration here. Keersmakecher and colleagues did whole exome sequencing on 67 T-ALL samples. They identified over 3,000 mutations of various types that affected the proteins encoded in 508 genes. They then used various strategies to sort through the genes and winnow them down to a manageable number of presumed driver genes. Of the final 15 genes they come up with, 8 had been identified previously as driver genes in T-ALL pathogenesis; an encouraging result. That left 7 new candidate driver genes, two of which encoded ribosomal proteins, thereby providing the rationale for discussing this manuscript in a DBA Newsletter. The two ribosomal protein genes are *RPL5*, a gene well-known to us interested in DBA, and *RPL10*, one of the 70 or so additional ribosomal protein genes that have yet to be implicated in DBA.

The nature of the mutations identified in *RPL10* and *RPL5* in this study are enlightening; again bringing to the forefront the concept that not all mutations are equal. I'll begin with Rpl10. We know very little about this protein other than that it is one of about 40+ proteins of the large ribosomal subunit. The *RPL30* mutations identified in this study showed a significant bias toward being found in the pediatric population examined, representing 8.2% of the pediatric cases. Of interest, all of the mutations identified in *RPL30* were missense mutations, where one amino acid was substituted for the other in the Rpl30 protein. Of considerable importance, virtually all of the substations occurred at one position in the Rpl10 protein. The finding that all but one of the T-ALL-associated mutations in *RPL10* affected the same residue, and that the amino acid replacing arginine at this position was almost always serine, strongly suggests some type of selection for this change in the outcome measured, which in this case was T-ALL. This in my mind is the hallmark of a likely driver mutation, a highly specific type of mutation that associates with disease phenotype. An examination of ribosome synthesis in cells from a patient with one of these *RPL10* mutations revealed a modest effect on polysome profiles a composite of effects on ribosome synthesis and function. One possibility here then is that in contrast to DBA where ribosomal proteins genes have general loss of function mutations that frequently occur throughout a gene, the very specific nature of the mutations in *RPL10* suggests that these mutations may subtly alter Rpl10 function in manner that somehow provides a driving force for the outgrowth of T-ALL tumors in certain patients. This subtle change in Rpl10 function could conceivably alter the synthesis of one or more oncogenes or tumor suppressor genes which play more direct role in promoting carcinogenesis.

The reason *RPL10* has not been identified as a DBA gene to date could be that it is found on the X chromosome and so general loss of function mutations such as those found in other DBA-related ribosomal protein genes would leave cells without a source of ribosomes and so be incompatible with life. So, the bottom line here is that very specific type of mutations in *RPL10* has been identified as a possible driver in T-ALL, which, while important for leukemogenesis, at this point in time seems to have relatively limited significance for the DBA community.

The issue becomes somewhat less clear with *RPL5*, the other ribosomal protein gene identified in the Keersmaecker study. *RPL5* is a known DBA gene (Gazda et al, 2008). It is mutated in about 9% of the DBA patients. Moreover, the mutations in *RPL5* found in the T-ALL patients included missense, frameshift, and nonsense mutations distributed throughout the protein sequence; with the latter two classes of mutations likely to truncate the protein and so have severe effects on function. Thus, it is likely that *RPL5* mutations found in the T-ALL cohort are general loss of function mutations similar to the type of mutations found in DBA patients.

So one question that comes to mind is why patients in the T-ALL cohort do not have DBA. The answer to this is relatively straightforward. The *RPL5* mutations in these cases were not congenital and found in every cell in the body but instead were somatic mutations which presumably arose in a T-cell progenitor that gave rise to the tumor cell population. Thus, erythroid progenitor cells lack these mutations and erythropoiesis was not affected, so no DBA. One could flip this around however, and then ask, why don't DBA patients with mutations in *RPL5* have T-ALL, since wouldn't all T cell progenitors in a DBA patient have the *RPL5* mutation? This is a more difficult question to answer. While the recent publication by Vlachos et al indicates patients with DBA have an increased risk relative to the general population

(Vlachos et al, 2012), there have been no reported cases of T-ALL in North American DBA Registry database despite having two patients with *RPL5* mutations with neoplasms. Thus, from this perspective having a congenital mutation in *RPL5* does not appear to strongly predispose a person to T-ALL. Perhaps then if *RPL5* is somehow involved in T-ALL it plays a more secondary or tertiary role; not so much as the driver in the front seat with the steering wheel in hand and foot on the accelerator but more of a back seat driver making suggestions, but without someone in the driver's seat to carry out these suggestions, the suggestions would have little impact where the car travels, or giving up on the analogy, cell growth or division decisions. New applications of "deep" sequencing can now distinguish driver from passenger mutations. Thus the role of RP mutations in cancer may ultimately be determined. Thus it is really too soon now to say exactly how mutations in *RPL10* or *RPL5* contribute to T-ALL.

We think at this point the implications of these studies on DBA are limited and do not substantially alter the recommendations in the Vlachos paper that patients with DBA receive general counseling regarding their cancer risk and that surveillance strategies for neoplastic complications need to be developed. It should be noted however, that without the conceptual framework created by studies on DBA it is unlikely that the results from the T-ALL study would carry the weight they do in terms potential involvement of ribosomal proteins in human diseases.

De Keersmaecker K, Atak ZK, Li N, Vicente C, Patchett S, Girardi T, Gianfelici V, Geerdens E, Clappier E, Porcu M, Lahortiga I, Luca R, Yan J, Hulselmans G, Vranckx H, Vandepoel R, Sweron B, Jacobs K, Mentens N, Wlodarska I, Cauwelier B, Cloos J, Soulier J, Uyttebroeck A, Bagni C, Hassan BA, Vandenberghe P, Johnson AW, Aerts S, Cools J (2013) Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. *Nature genetics* **45:** 186-190

Gazda HT, Sheen MR, Vlachos A, Choesmel V, O'Donohue MF, Schneider H, Darras N, Hasman C, Sieff CA, Newburger PE, Ball SE, Niewiadomska E, Matysiak M, Zaucha JM, Glader B, Niemeyer C, Meerpohl JJ, Atsidaftos E, Lipton JM, Gleizes PE, Beggs AH (2008) Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond-Blackfan anemia patients. *American journal of human genetics* **83:** 769-780

Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM (2012) Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. *Blood* **119:** 3815-3819

Wong SF, Mirshahidi H (2011) Use of tyrosine kinase inhibitors for chronic myeloid leukemia: management of patients and practical applications for pharmacy practitioners. *The Annals of pharmacotherapy* **45:** 787-797