No man is an island,

or so sayeth John Donne, a Jacobean poet and preacher. I'm sure I am not original in my view that what Donne was speaking of with this phrase: most certainly the concept that no person is independent of the influence of others or the influence that they may have on others.

The same could be said of genes. Genes are not found floating in the nucleus of cells as independent entities. Nay, they are found as part of chromosomes, 46 of which are found in the nucleus of most cells in our body. Each chromosome is composed of two strands of DNA winding about one another, one heading left to right and the other right to left (so to speak). These strands of DNA are composed of deoxyribonucleotides linked to one another through phosphodiester bonds. There are millions, sometimes hundreds of millions, of deoxyribonucleotide units per strand of DNA in each of our chromosomes.

Within these hundreds of millions of deoxyribonucleotide units is information that codes for the tens of thousands of proteins residing in the human genome. This information is stored within the sequence of the four-deoxyribonucleotide bases that make up DNA (GATC). As has been discussed in this forum previously, each of these genes contains additional information (also embodied in the base sequence) needed for the appropriate expression of each gene. By appropriate, I mean that the gene is expressed in the correct cell type, at a fitting time in development, and in just the right amounts.

When one cell becomes two during cell division, all this genetic information spread across 3.5 billion base pairs of DNA needs to be faithfully copied with one genetic blueprint retained in the original mother cell and the second transmitted to the newly created daughter cell. This segregation of genetic information between mother and daughter cell is made much easier by having our genes packaged in 46 chromosomes as opposed to having to distribute exact copies of tens of thousands of individual genes between mother and daughter cells during each cell division.

These requisites for faithful transmission of genetic information from one cell to another bring us to this month's *Journal Club*. The manuscript I'll be discussing is from the laboratory of Stefan Karlsson, an established investigator in the DBA field who has had a longstanding interest in using gene therapy as a cure for DBA. The title of the article is **"Gene therapy cures the anemia and lethal bone marrow failure in a mouse model for RPS19-deficient Diamond Blackfan anemia"** [1].

In previous reports, the Karlsson laboratory has described a mouse model of DBA where expression of the Rps19 protein is placed under the control of a drug that can be included in the drinking water. Depending on the particular mouse strain used, this drug can induce a mild anemia or a lethal bone marrow failure depending on the degree to which levels of Rps19 are reduced. In the current manuscript, they rescue phenotypes associated with reduced expression of Rps19 using gene therapy. As exciting as these results are, they are tempered by lingering safety concerns regarding the use gene therapy to correct genetic disorders in humans.

For gene therapy to be effective, a delivered gene must be incorporated into one of the 46 chromosomes so that it can be faithfully transmitted from mother to daughter cell during cell division. **While the gene used by Karlsson and co-workers as a proof of principle was RPS19, it should be noted at this point that this approach should be readily amenable to any of the known genes affected by loss of function mutations in DBA patients.** To deliver any gene used for gene therapy, the gene is first incorporated into human viral vector, which is adapted from a human virus where part of the virus's life cycle is to incorporate itself into an infected cell's chromosome. These viral vectors are modified in such a way that most of the information needed for a productive viral infection has been removed, thereby reducing safety concerns in this regard.

Where the current concerns regarding gene therapy safety reside is not in the viral vectors themselves but instead on where they are incorporated into the chromosomes of the cells they enter and their influence on the expression of resident genes near their sites of integration. Gene therapy trials in the mid-2000's were set back when several patients treated for an X-linked severe combined immunodeficiency disorder developed leukemia [2]. These leukemias were caused by the insertion of the viral vector near proto-oncogenes in the recipient's genome.

Proto-oncogenes are normal genes that when hyper-activated either by a mutational change that increases activity, compromises normal regulation, or simply increases expression causes that gene to override normal controls on cell proliferation and cause cancer. Insertion of viral vectors used in gene therapy near such proto-oncogenes can lead to their activation as oncogenes and so put a patient receiving gene therapy at risk for cancer. This concern is heightened in studies like those described in the Karlsson manuscript where the promoter used to drive RPS19 expression was very strong and is expressed in virtually all cell types. Such promoters could work over relatively large distances and so activate genes nearby the site of integration.

At present it is difficult to control where viral vectors used in gene therapy will insert in chromosomes. Further these vectors integrate at multiple sites within a cell's genome increasing the risk that an inadvertent insertional event could have unanticipated negative outcomes. The Karlsson study examined the integration profile of their viral vectors and identified many sites within the mouse genome where their vector integrated. The results suggest that viral integration is not random, apparently favoring some sites in the genome over others. Importantly, they did not detect insertions near possible proto-oncogenes nor did they see any hematological abnormalities indicative of a pre-leukemic state.

While the studies from the Karlsson laboratory using gene therapy to rescue phenotypes in their mouse model of DBA are encouraging, more work is needed in human systems before this approach can be translated into DBA patients. Gene therapy trials continue for human diseases were the risks of gene therapy are offset by the severity of the disease. One such trial for adenosine deaminase deficiency in human patients has shown positive results without evidence leukemic complications [3]. The small numbers of patients receiving such therapies at present make it difficult to quantify risk within a particular patient population and even more difficult to extrapolate risks to other patient groups. **Nevertheless, the continued development of viral vectors for gene therapy in treating a number of different human genetic diseases should help pave the way for pioneers like Stefan Karlsson who are adapting these advances for use in DBA patients.**

[1] Jaako, P., Debnath, S., Olsson, K., Modilch, U., Rothe, M., Schambach, A., Flygare, J., and Karlsson, S. (2014) Gene Therapy cures the anemia and lethal bone marrow failure in mouse model for RPS19-deficient Diamond Blackfan anemia. *Hematologica* E-pub ahead of print

[2] Hacein-Bey-Abina, H, *et al* (2008) Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *JCI* **118**, 3132-3142

[3] Candotti, F. *et al* (2012) Gene therapy for adenosine deaminase-deficient severe combined immune deficiency: Clinical comparison of retroviral vectors and treatment plans. *Blood* **120**,3635-3646