Intramural papers of the month

By Jacqueline de Marchena, Monica Frazier, Raj Gosavi, and Bailey Schug

Understanding how ribonucleotides in DNA exert their biological effects

NIEHS scientists recently showed that a surprisingly large number of ribonucleotides are incorporated into the genome during nuclear DNA replication. They have now shown that if these ribonucleotides are not removed from the genome, they cause replicative DNA polymerases to stall during subsequent rounds of replication. This stalling increases as the number of consecutive ribonucleotides present in the DNA template increases from one to four.

Structural studies using X-ray crystallography reveal that the presence of ribonucleotides in the DNA template is associated with changes in the conformation of both the DNA and the replicative polymerase, and that the extent of these changes correlates with the amount of stalling. This work enhances our understanding of the biological consequences of ribonucleotides in DNA. Their effects may actually be beneficial in some circumstances, but, in other circumstances, they may result in replication stress and genome instability. These latter, negative effects may be relevant to diseases associated with defects in the processes that remove ribonucleotides from the genome, including a rare neuroinflammatory condition, Aicardi-Goutie`res syndrome. **(RG)**

Citation: Clausen AR, Murray MS, Passer AR, Pedersen LC, Kunkel TA.
(http://www.ncbi.nlm.nih.gov/pubmed/24082122)
2013. Structure-function analysis of ribonucleotide bypass by B family DNA replicases. Proc Natl Acad Sci U S A 110(42):16802-16807.

Stress hormone signaling found to prevent heart disease

Recently reported in PNAS, NIEHS researchers and their collaborators discovered that glucocorticoid receptor (GR) signaling in cardiomyocytes is necessary for normal heart function, and may provide a therapeutic approach for treating cardiovascular disease.

Chronic stress is increasingly recognized for its contribution to the development and progression of heart disease, a leading cause of death in the Western world. Glucocorticoids are hormones that mediate the stress response by binding the GR and regulating gene expression. To determine the role of stress hormone signaling in heart muscle cells, the researchers generated knockout mice lacking GR specifically in cardiomyocytes.

They found that these mice die early from spontaneous cardiovascular disease. By three months of age, the mice exhibited cardiac hypertrophy and a decrease in left ventricular systolic function. Hearts became severely dilated by six months of age, resulting in premature death. Global gene expression analysis of GR-deficient hearts revealed a decrease in the expression of genes that play an important role in maintaining cardiac contractility, repressing cardiac hypertrophy, promoting cardiac survival, and inhibiting inflammation. Therefore, while sustained increases in glucocorticoids have been associated with adverse effects on the heart, these results suggest that the normal pulsatile secretion of these hormones is critical for maintaining cardiovascular function. **(JDM)**

Citation: Oakley RH, Ren R, Cruz-Topete D, Bird GS, Myers PH, Boyle MC, Schneider MD, Willis MS, Cidlowski JA.
(http://www.ncbi.nlm.nih.gov/pubmed/24082121)
2013. Essential role of stress hormone signaling in cardiomyocytes for the prevention of heart disease. Proc Natl Acad Sci U S A 110(42):17035-17040.

DNA methylation as a biomarker to detect breast cancer

Sister Study researchers at NIEHS have shown that the overall level of DNA methylation across the genome is associated with future risk of developing cancer. DNA methylation is a form of epigenetic modification associated with gene expression.

The Sister Study is designed to explore genetic and environmental determinants of breast cancer. The NIEHS team measured methylation at LINE-1 elements in blood DNA samples collected from some women who later developed breast cancer, and

compared their methylation levels to women in the study who remained cancer-free. They found that women with low levels of blood DNA methylation had a 1.75-fold increased risk of developing breast cancer compared to women with high levels of methylation, and that these differences were detectable months to years before the clinical diagnosis of breast cancer.

DNA methylation changes in response to environmental exposures, such as chemical pollutants, diet, and other lifestyle factors, which in turn may be associated with an increased risk of cancer. This study adds to the growing body of literature linking changes in blood DNA methylation to future risk of developing cancer, and may help researchers move closer to developing blood tests that can predict a woman's chance of getting cancer, as well as lifestyle strategies for lowering risk. **(BS)**

Citation: DeRoo LA, Bolick SC, Xu Z, Umbach DM, Shore D, Weinberg CR, Sandler DP, Taylor JA. *(http://www.ncbi.nlm.nih.gov/pubmed/24130171)*
2013. Global DNA methylation and one-carbon metabolism gene polymorphisms and the risk of breast cancer in the Sister Study. Carcinogenesis; doi:10.1093/carcin/bgt342 [Online 15 October 2013].

Tanning gene linked to increased risk of testicular cancer

Collaborative efforts between NIEHS and the University of Oxford have found that a variant in a gene that promotes skin tanning is associated with an increased risk of testicular cancer. This variant, also known as a single-nucleotide polymorphism (SNP), is located in the KITLG gene, a gene that is controlled by the tumor suppressor p53, the most commonly mutated gene in human cancers.
The group combined searches of comprehensive data sets of genetic variation associated with cancer risk with laboratory experiments, and identified a single SNP that affects p53 binding to a control element in the KITLG gene. The SNP confers protection to the skin against sun damage, by increasing production of pigmented cells called melanocytes. The authors suggest that during human evolution, this SNP has become more common in populations with light skin for this beneficial reason.

However, in testicular cells, the situation appears reversed. The researchers hypothesize that increased cell division driven by the KITLG SNP may permit the growth of tumor cells, increasing cancer risk for those that carry the gene variant. Interestingly, for this type of tumor growth to occur, the p53 gene must remain fully functional, which is very unusual in cancers, and this may explain why testicular cancers have a very high cure rate. **(MF)**

Citation: Zeron-Medina J, Wang X, Repapi E, Campbell MR, Su D, Castro-Giner F, Davies B, Peterse EF, Sacilotto N, Walker GJ, Terzian T, Tomlinson IP, Box NF, Meinshausen N, De Val S, Bell DA, Bond GL. *(http://www.ncbi.nlm.nih.gov/pubmed/24120139)*
2013. A polymorphic p53 response element in KIT ligand influences cancer risk and has undergone natural selection. Cell 155(2):410-422. Story

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