

Intramural papers of the month

By Greg Buchold, Tara Ann Cartwright, Geoffrey Feld, Deepa Singh, and Qing Xu

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NTP researchers demonstrate common drinking water disinfectant is carcinogenic

Several haloacetic acids are already regulated by the U.S. Environmental Protection Agency as carcinogens, and this National Toxicology Program study argues that bromodichloroacetic acid (BDCA) should also be considered a potential human environmental health hazard. The authors say that BDCA is one of the most common byproducts of water disinfection, formed by the reaction of oxidizing agents containing chlorine with naturally occurring organic material and bromide ions in source water. They estimated that exposure risk to the public could be substantial.

Exposing rats to BDCA caused the animals to develop a hormone-dependent (ERalpha+/PR+) mixed ductal-basal breast cancer. The malignancy was similar to human breast cancer, displaying several distinct molecular features, indicating a more aggressive capacity compared to the spontaneous breast tumors that arose in the untreated animals.

The chemical produced mutations in genes frequently altered in human breast cancer, including p53, PTEN, and EGFR. The tumors also showed increased levels of the hereditary breast cancer gene BRCA2 and TGFbeta. Notably, gene expression changes in the tumors indicated a cluster of genes likely elevated by the increased TGFbeta signaling, promoting new blood vessel growth (VEGFA), degradation of the extracellular matrix (MMP9, MMP2, THBS1, ID1), and epithelial-to-mesenchymal transition (TWIST), changes that promote cancer growth and invasion. **(GB)**

Citation: Harvey JB, Hong HH, Bhusari S, Ton TV, Wang Y, Foley JF, Peddada SD, Hooth M, DeVito M, Nyska A, Pandiri AR, Hoenerhoff MJ. (<http://www.ncbi.nlm.nih.gov/pubmed/25732176>)

F344/NTac rats chronically exposed to bromodichloroacetic acid develop mammary adenocarcinomas with mixed luminal/basal phenotype and Tgfbeta dysregulation. *Vet Pathol*; doi:10.1177/0300985815571680 [Online 2 March 2015].

NELF mediated Pol II pausing in ESCs controls signaling pathways necessary for development

A paradigm got a makeover as scientists at NIEHS reported that plasticity of embryonic stem cells (ESCs) is facilitated by pausing of RNA polymerase II (Pol II). Surprisingly, pausing did not occur on developmental genes, as has been observed in *Drosophila* embryos. Instead, it occurred on genes regulating cell cycle and signal transduction. The study improves understanding of mechanisms that underlie the pluripotency of stem cells, which is necessary for the development of regenerative medicines.

Researchers used high-resolution genomic analysis to investigate distribution of paused Pol II and gene expression in ESCs that were specifically maintained in a nondifferentiated state. The finding highlights that Pol II pausing is enriched at cell cycle regulators and at genes involved in signal transduction. Moreover, this observation was consistent in ESCs studied in different conditions and methodologies. To evaluate the functional significance of Pol II pausing, the authors genetically deleted the pause-inducing factor, NELF. In the absence of NELF, ESCs were resistant to differentiation through inhibition of key developmental signaling pathways. Together these studies contribute to a rapidly developing potential for ESC reprogramming for the benefit of human health. **(DS)**

Citation: Williams LH, Fromm G, Gokey NG, Henriques T, Muse GM, Burkholder A, Fargo DC, Hu G, Adelman K. (<http://www.ncbi.nlm.nih.gov/pubmed/25773599>)

Pausing of RNA polymerase II regulates mammalian developmental potential through control of signaling networks. *Mol Cell* 58(2):311–322. [Story]

Genetic and environmental factors interact to affect the severity of infant RSV bronchiolitis

NIEHS researchers and collaborators have discovered that living conditions and toll-like receptor 4 (TLR4) genotypes interact in infants to determine the consequence of respiratory syncytial virus (RSV) infection. The findings open new avenues for interventions in RSV disease.

Severe RSV infection is the leading cause of infant hospitalization worldwide. Activation of TLR4, an endotoxin lipopolysaccharide (LPS)-sensing receptor, has been implicated in RSV pathogenesis. The researchers studied hospitalized babies with RSV bronchiolitis in rural and urban regions of Argentina. They found that among rural children with high LPS exposure, TLR4 mutations correlating with reduced response to LPS are found more frequently in mild cases, but in urban children with low LPS exposure, the mutations are found more frequently in severe cases.

Interestingly, RSV titers were not associated with disease severity or the interaction between TLR4 and LPS. Patients from rural homes had suppressed inflammatory cytokines, suggesting environmental conditioning of the immune system. In addition, high GATA3/T-bet and IL-4/IFNgamma ratios, indicators of Th2 polarization, are present in severe RSV cases. The researchers also confirmed these findings in a mouse model of RSV bronchiolitis. Molecules identified in this study could help develop better treatments for illness from RSV. **(QX)**

Citation: Caballero MT, Serra ME, Acosta PL, Marzec J, Gibbons L, Salim M, Rodriguez A, Reynaldi A, Garcia A, Bado D, Buchholz UJ, Hijano DR, Coviello S, Newcomb D, Bellabarba M, Ferolla FM, Libster R, Berenstein A, Siniawski S, Blumetti V, Echavarria M, Pinto L, Lawrence A, Ossorio MF, Grosman A, Mateu CG, Bayle C, Dericco A, Pellegrini M, Igarza I, Repetto HA, Grimaldi LA, Gudapati P, Polack NR, Althabe F, Shi M, Ferrero F, Bergel E, Stein RT, Peebles RS, Boothby M, Kleeberger SR, Polack FP. (<http://www.ncbi.nlm.nih.gov/pubmed/25555213>)

2015. TLR4 genotype and environmental LPS mediate RSV bronchiolitis through Th2 polarization. *J Clin Invest* 125(2):571–582. [Story]

Leading the way in ribonucleotide excision repair

NIEHS researchers and colleagues reveal how the enzyme topoisomerase 1 (Top1) mediates ribonucleotide excision repair (RER) only on the leading strand and not the lagging strand during DNA replication. The erroneous incorporation of ribonucleotides into the genome overwhelmingly represents the most common form of DNA damage, often resulting in several mutagenic consequences, including the autoimmune disorder Aicardi-Goutieres syndrome.

Using a yeast model, the team previously reported on a mutant variant of the DNA polymerase responsible for leading strand extension, pol2-MG, which incorporates high levels of ribonucleotides into DNA. This construct enabled the identification of RNase H2 as the critical enzyme responsible for removing the incorporated ribonucleotides. In this study, the authors examined lagging strand polymerase variants, pol1-LM and pol3-LM, using yeast genetics, radiolabeling, and a new method developed in their lab, called hydrolytic 5'-DNA end-sequencing, or HydEn-seq. The pol1-LM and pol3-LM enzymes also incorporate ribonucleotides into genomes, and these ribonucleotides are removed in an RNase H1-dependent manner.

Top1 also catalyzes RER activity, but requires additional post-processing that often leaves the genome susceptible to further damage. When RER-defective yeast strains lacking Top1 are considered, only pol2-MG, not pol1-LM or pol3-LM, results in an increase in DNA damage. The authors propose three, non-exclusive testable models to explain Top1's preference for leading strand RER, and future work is geared towards pinning down the correct model. **(GF)**

Citation: Williams JS, Clausen AR, Lujan SA, Marjavaara L, Clark AB, Burgers PM, Chabes A, Kunkel TA.

(<http://www.ncbi.nlm.nih.gov/pubmed/25751426>)

2015. Evidence that processing of ribonucleotides in DNA by topoisomerase 1 is leading-strand specific. *Nat Struct Mol Biol* 22(4):291-297.

Inhibition of NADPH oxidase halts disease progression in Parkinson's disease models

Scientists from the NIEHS Neurobiology Laboratory and the National Toxicology Program showed that post-treatment of an ultra-low dose of the nicotinamide adenine dinucleotide phosphate (NADPH) inhibitor, diphenyleneiodonium (DPI), reduces both neuroinflammation and neurotoxicity in rodent Parkinson's disease (PD) models by systemic injections of either endotoxin lipopolysaccharide (LPS) or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Since PD is a progressive disabling disorder characterized by dopaminergic degeneration, the findings provide a promising therapeutic strategy for stopping the progression of the disease and other neurodegenerative disorders.

The study, published in the journal *Brain*, demonstrated that subcutaneous infusion of ultra-low dose DPI to both LPS and MPTP rodent models reduced microglia-mediated chronic neuroinflammation by selectively inhibiting NADPH oxidase activation. Inhibiting NADPH oxidase attenuated the progression of dopaminergic neuron degeneration and motor deficits. Additionally, mice treated with ultra-low dose DPI did not display overt organ toxicity or any effects on body weight. Results also indicated that DPI in low concentrations did not influence peripheral immune cell function. Taken together, these findings show that ultra-low dose DPI is an excellent drug candidate for future clinical trials. **(TAC)**

Citation: Wang Q, Qian L, Chen SH, Chu CH, Wilson B, Oyarzabal E, Ali S, Robinson B, Rao D, Hong JS.

(<http://www.ncbi.nlm.nih.gov/pubmed/25716193>)

2015. Post-treatment with an ultra-low dose of NADPH oxidase inhibitor diphenyleneiodonium attenuates disease progression in multiple Parkinson's disease models. *Brain* 138(Pt 5):1247-1262.

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