

## Intramural papers of the month

By Tara Ann Cartwright, Monica Frazier, Jacqueline Powell, Jordan St. Charles, and Qing Xu

- [A comparison of the effectiveness of RNA-seq and microarrays for clinical and toxicological applications](#)
- [NADPH oxidase implicated in Parkinson's disease-associated substance P neurotoxicity](#)
- [Nrf2 polymorphisms correlate with differential susceptibility to acute lung injury](#)
- [MMS exposure is associated with mtDNA mutagenesis](#)
- [Scavenger receptor B-I plays essential role in immune response to pneumonia](#)

### **A comparison of the effectiveness of RNA-seq and microarrays for clinical and toxicological applications**

In a recent collaboration with the MicroArray Quality Control (MAQC) consortium, led by the U.S. Food and Drug Administration, National Toxicology Program and NIEHS researchers found that, depending on the treatment effect, RNA-seq and microarrays can similarly detect expression changes in rat livers treated with one of 27 different chemicals with varying mechanisms of action. Microarrays have been a staple to detect differentially expressed genes and to predict toxicity outcomes of new drug treatments. RNA-seq technology is being adopted by researchers for use in a variety of gene expression analyses. This study was part of the sequencing quality control phase of MAQC, which rigorously tests emerging technologies, ensuring they are reliable for these applications.

The researchers determined as long as the agents used induced a large enough transcriptional response, both RNA-seq and microarrays detected a similar set of differentially expressed genes, although RNA-seq was slightly better at detecting changes and agreed more with external validation. However, when the treatment agent did not induce a large effect, there were discrepancies between the two data sets that resulted from genes with below-median expression. In comparison with previous studies that have examined similar questions, this study examined differences in detection between the two methods with a large variety of treatment conditions.

The authors suggest that RNA-seq may be used instead of microarray experiments when comparing samples with similar biological conditions. However, microarrays or RNA-seq may be equally effective when developing genomic biomarker tests.

**(JS)**

*Citation:* Wang C, Gong B, Bushel PR, Thierry-Mieg J, Thierry-Mieg D, Xu J, Fang H, Hong H, Shen J, Su Z, Meehan J, Li X, Yang L, Li H, Labaj PP, Kreil DP, Megherbi D, Gaj S, Caiment F, van Delft J, Kleinjans J, Scherer A, Devanarayan V, Wang J, Yang Y, Qian HR, Lancashire LJ, Bessarabova M, Nikolsky Y, Furlanello C, Chierici M, Albanese D, Jurman G, Riccadonna S, Filosi M, Visintainer R, Zhang KK, Li J, Hsieh JH, Svoboda DL, Fuscoe JC, Deng Y, Shi L, Paules RS, Auerbach SS, Tong W. (<http://www.ncbi.nlm.nih.gov/pubmed/25150839>)

2014. The concordance between RNA-seq and microarray data depends on chemical treatment and transcript abundance. *Nat Biotechnol* 32(9):926-932.

### **NADPH oxidase implicated in Parkinson's disease-associated substance P neurotoxicity**

Scientists in the NIEHS Neurobiology Laboratory have discovered the first evidence that the endogenous peptide, substance P, utilizes a neurokinin-1 receptor independent pathway of neurotoxicity at subpicomolar concentrations, mediated by NADPH oxidase. Substance P, produced in neurons, amplifies proinflammatory responses in the central nervous system. This type of response has been implicated in Parkinson's disease pathogenesis, because it leads to loss of dopaminergic neurons and motor behavior control.

The researchers noted that previous studies have suggested substance P toxicity is both dependent and independent of the substance P receptor, neurokinin-1. They hypothesized that different signaling pathways were at work at different substance P concentrations.

To test their hypothesis, the researchers treated mice and primary cell cultures with two toxins that cause dopaminergic neurodegeneration, and found that substance P toxicity is mediated by activation of microglial NADPH oxidase at extremely low concentrations, independent of neurokinin-1. Further, they found that at high substance P concentrations, toxicity is dependent on neurokinin-1. Interestingly, intermediate concentrations showed decreased toxic effects, further pointing to bimodal mechanisms of substance P toxicity. In particular, their novel discovery of NADPH oxidase's role in mediating substance P's effects may lead to new insights in Parkinson's disease pathogenesis. **(MF)**

*Citation:* Wang Q, Chu CH, Qian L, Chen SH, Wilson B, Oyarzabal E, Jiang L, Ali S, Robinson B, Kim HC, Hong JS. (<http://www.ncbi.nlm.nih.gov/pubmed/25209287>)

2014. Substance P exacerbates dopaminergic neurodegeneration through neurokinin-1 receptor-independent activation of microglial NADPH oxidase. *J Neurosci* 34(37):12490-12503.

## **Nrf2 polymorphisms correlate with differential susceptibility to acute lung injury**

NIEHS researchers have identified single nucleotide polymorphisms (SNPs) of Nrf2 in diverse mice strains, and have associated these SNPs with differential susceptibility to acute lung injury. The findings help better understand the role of Nrf2 polymorphisms in oxidative lung disorders.

Nrf2 is a master transcriptional factor that mediates induction of a variety of genes involved in antioxidant defense. Previous studies using mice with simple deletion of Nrf2 have given limited information about its function and regulation.

By compiling information from public databases and resequenced data, the researchers were the first to discover more than a thousand Nrf2 SNPs across multiple inbred mouse strains. After exposing the strains that were grouped into three SNP haplotypes to lung-damaging hyperoxia, or excess oxygen, they observed that haplotype 2 and haplotype 3 strains were more susceptible to hyperoxia-induced injury than haplotype 1 strains. Therefore, Nrf2 haplotypes significantly correlated with hyperoxia susceptibility.

The team also determined the functional effects of Nrf2 SNPs on the expression and activity of Nrf2, which may contribute to the difference in susceptibility. Since many genetic mutations of Nrf2 are found in human oxidative disorders, mouse strains characterized by Nrf2 haplotypes in this work provide useful tools to learn how Nrf2 is involved in these diseases. **(QX)**

*Citation:* Cho HY, Jedlicka AE, Gladwell W, Marzec J, McCaw ZR, Bienstock R, Kleeberger SR. (<http://www.ncbi.nlm.nih.gov/pubmed/25268541>)

2014. Association of Nrf2 polymorphism haplotypes with acute lung injury phenotypes in inbred strains of mice. *Antioxid Redox Signal*; doi:10.1089/ars.2014.5942 [Online 12 November 2014].

## **MMS exposure is associated with mtDNA mutagenesis**

Researchers from the NIEHS Genome Integrity and Structural Biology Laboratory have identified a novel genetic and environmental interaction which alters mitochondrial DNA (mtDNA) replication efficiency. Maintenance of mtDNA is essential for cellular survival in eukaryotic cells, and the inability to properly replicate results in mitochondrial disease states and toxicity. Scientists investigated mtDNA replication efficiency, by examining yeast strains previously characterized with mutations in the mtDNA polymerase gene, in the presence of the alkylating base damaging agent, methyl methanesulfonate (MMS).

The study, published in *PLOS Genetics*, demonstrated that MMS exposure increased mtDNA mutagenesis in strains with disease-associated mutations that disrupt polymerase activity. Approximately half of the mutations arising from MMS exposure were cytosine to guanine transversions. Further observations suggested that MMS-induced mutagenesis did not arise by disrupting exonuclease activity.

Mechanistically, the authors have demonstrated that MMS exposure induced mtDNA mutagenesis in single-stranded mtDNA. Furthermore, this study supports a polymerase switching mechanism in mtDNA replication, which exposes single-stranded DNA to mutagenesis that was previously not described. Since the current study was conducted in yeast, it raises the question whether a similar mechanism occurs in mammalian mtDNA, and offers new insights for patients suffering from mitochondrial diseases and their susceptibility to DNA damaging agents. **(TAC)**

*Citation:* Stumpf JD, Copeland WC.

(<http://www.ncbi.nlm.nih.gov/pubmed/25340760>)

2014. MMS exposure promotes increased mtDNA mutagenesis in the presence of replication-defective disease-associated DNA polymerase gamma variants. *PLoS Genet* 10(10):e1004748.

## **Scavenger receptor B-I plays essential role in immune response to pneumonia**

NIEHS researchers recently discovered that the scavenger receptor B-I (SR-BI), which has an environmentally regulated level of expression, is critical to survival during bacterial pneumonia.

After the scientists inoculated SR-BI deficient mice with the bacterium *Klebsiella pneumoniae*, the mice suffered markedly increased mortality compared to controls. The SR-BI deficient mice also had significantly elevated levels of bacteria in the lung, blood, and liver following inoculation, suggesting a deficit in both the innate immune response of the lung, as well as the host defense response throughout the body.

Because these increased bacteria levels were accompanied by sustained elevations of neutrophils and neutrophil-recruiting

cytokines in the airway, the investigators examined whether SR-BI may mediate clearance of bacterial lipopolysaccharide (LPS) from the airspace. SR-BI deficient macrophages were, in fact, defective in clearing LPS from the lung, and also produced elevated levels of proinflammatory cytokines in response to LPS. The investigators also found that SR-BI deficient mice had defective production of adrenal stress steroid hormones during pneumonia, and this deficit in adrenal steroids also enhanced migration of neutrophils into the lung. Despite the increase in neutrophils, bacterial killing function was defective in SR-BI deficient neutrophils, compromising host defense.

These findings suggest that SR-BI plays an important role in guiding macrophages to clear LPS from the airway, and in regulating neutrophil migration to the lung via dual effects on innate cytokine production and adrenal function. Taken together, the researchers have uncovered an essential new role for SR-BI in the integrated physiologic response to pneumonia and in phagocyte antimicrobial function. **(JP)**

*Citation:* Gowdy KM, Madenspacher JH, Azzam KM, Gabor KA, Janardhan KS, Aloor JJ, Fessler MB.

(<http://www.ncbi.nlm.nih.gov/pubmed/25336169>)

2014. Key role for scavenger receptor B-I in the integrative physiology of host defense during bacterial pneumonia. *Mucosal Immunol*; doi:10.1038/mi.2014.88 [Online 22 October 2014].

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