Intramural papers of the month

By Tara Ann Cartwright, Monica Frazier, Simone Otto, Qing Xu, and Annah Wyss

- NTP helps develop technique for detecting chemicals that disrupt testis function
- Pesticide use may increase depression risk among farmers
- DNA replication errors in yeast offer new insight into cancer research
- Genistein exposure alters the action of glucocorticoids
- Orphan receptor regulates lipid metabolism

NTP helps develop technique for detecting chemicals that disrupt testis function

Scientists from the National Toxicology Program and the U. S. Environmental Protection Agency have developed and validated a screening process to identify potential disruptors of fetal testosterone synthesis and testis gene expression *in utero*. Called the Fetal Phthalate Screen, the protocol identifies causes of phthalate syndrome, a condition in which exposure to phthalate esters (PEs) decreases testosterone production during genital development, resulting in abnormalities after birth.

PEs are found in a variety of products used every day, including medical supplies, toys, and personal care products. The goal of this study was to help the EPA in their evaluation of PEs in products and the potential need for policy to control their use.

During the course of 2-3 years, 27 chemicals were assessed by measuring maternal rodent weight gain, fetal viability, and testis testosterone production levels *ex vivo* after PE exposure. The screen accurately quantitated the effects of PEs with known consequence, and also evaluated a series of chemicals for the first time.

Other methodologies exist for identifying chemicals that result in phthalate syndrome, but this screen does it more accurately with greater efficiency, and uses fewer animals than previous techniques. **(MF)**

Citation: Furr JR, Lambright CS, Wilson VS, Foster PM, Gray LE Jr *(http://www.ncbi.nlm.nih.gov/pubmed/24798384)* 2014. A short-term in vivo screen using fetal testosterone production, a key event in the phthalate adverse outcome pathway, to predict disruption of sexual differentiation. Toxiol Sci 140(2):403-424.

Pesticide use may increase depression risk among farmers

In a recent study, NIEHS scientists reported that farmers exposed to certain types of pesticides may have an increased risk for depression. Pesticides are hypothesized to affect neurotransmission, and several previous epidemiologic studies have found positive associations between use of pesticides and depression.

Using data from the Agricultural Health Study, the investigators examined use of 10 pesticide classes comprising 50 specific pesticides among a large cohort of 21,208 male licensed pesticide applicators, mainly farmers, from North Carolina and Iowa. Among this cohort, 19,506 never reported being diagnosed with depression by a physician, while 1,702 reported a physician diagnosis of depression at baseline or during follow-up.

Ever use of 2 classes of pesticides, fumigants and organochlorine insecticides, and 7 specific pesticides — the fumigants aluminum phosphide and ethylene dibromide; the phenoxy herbicide 2,4,5-T; and the insecticides dieldrin, diazinon, malathion, and parathion — were associated with elevated risk of depression. Risk of depression also appeared to increase with the number of cumulative days of any pesticide use, and was heightened for those ever diagnosed with pesticide poisoning or reporting an incident of unusually high personal pesticide exposure.

This large and comprehensive study supports previous research by indicating an elevated risk of depression among farmers who use pesticides, while highlighting classes and specific pesticides that may warrant follow-up in animal studies. **(AW)**

Citation: Beard JD, Umbach DM, Hoppin JA, Richards M, Alavanja MC, Blair A, Sandler DP, Kamel F *(http://www.ncbi.nlm.nih.gov/pubmed/24906048)* . 2014. Pesticide exposure and depression among male private pesticide applicators in the Agricultural Health Study. Environ Health Perspect 122(9):984-991.

DNA replication errors in yeast offer new insight into cancer research

Researchers at NIEHS have discovered new information regarding the rates of errors yeasts can make during DNA replication and the efficiency with which the mismatch repair system (MMR) can correct these errors. The findings could help better understand mutations in both evolution and human cancers and better identify mutations that drive cancer formation.

Starting from multiple genomic origins, DNA polymerases use existing DNA strands as templates during synthesis of new strands via serial addition of complementary nucleotides. Despite very high polymerase accuracy and MMR efficiency, errors slip through and cause mutations. Mutations and epigenetic changes in polymerase and MMR genes can dramatically increase both the risk of cancer and mutation rates in affected tumors.

By sequencing 70 nuclear genomes from 8 yeast strains with a variety of MMR and polymerase defects, researchers in this study collected an unprecedented 40,000 mutations. From observed error rates, they concluded that MMR corrects more than 99 percent of all mismatches. They found that MMR efficiency and replication fidelity are both influenced by mismatch type, the responsible polymerase, timing of replication, and replication origin proximity. Mutation rates also vary by replication fork direction, protein coding state, nucleosome proximity, and sequence context. Mutation patterns established in this work explain patterns in hypermutated endometrial cancers. **(QX)**

Citation: Lujan SA, Clausen AR, Clark AB, MacAlpine HK, MacAlpine DM, Malc EP, Mieczkowski PA, Burkholder AB, Fargo DC, Gordenin DA, Kunkel TA
(http://www.ncbi.nlm.nih.gov/pubmed/25217194)
. 2014. Heterogeneous polymerase fidelity and mismatch repair bias genome variation and composition. Genome Res; doi:10.1101/gr.178335.114 [Online 12 September 2014].

Genistein exposure alters the action of glucocorticoids

Researchers from NIEHS have identified a novel mechanism by which genistein, an estrogen-like molecule naturally found in soy products, regulates glucocorticoid receptor (GR) mediated gene expression. Genistein competes with estradiol for estrogen receptor (ER) binding and is associated with reduced fertility and uterine pathologies. The findings appeared in Environmental Health Perspectives.

The authors used microarray analysis to show that genistein exposure induced a different transcriptional response in Ishikawa cells compared to estradiol. In fact, the genes regulated by genistein belong to distinct networks involved in biological pathways. The scientists observed a unique pattern of gene regulation in response to co-administration of genistein and the synthetic glucocorticoid dexamethasone compared to estradiol and dexamethasone.

They also demonstrated that genistein alters glucocorticoid regulation of GR-target genes and found that both GR and ERalpha, one of two specific estrogen receptors, were required to regulate a select set of genes by genistein and dexamethasone.

Taken together, the findings from this study provide an *in vitro* model for understanding the mechanistic actions of genistein. More importantly, the identification of co-regulated genes by microarray analysis provides novel molecular targets for future studies. **(TAC)**

Citation: Whirledge S, Senbanjo LT, Cidlowski JA
(http://www.ncbi.nlm.nih.gov/pubmed/25136773)
. 2014. Genistein disrupts glucocorticoid receptor signaling in human uterine endometrial Ishikawa cells. Environ Health Perspect; doi:10.1289/ehp.1408437 [Online 19 August 2014].

Orphan receptor regulates lipid metabolism

According to NIEHS scientists and their collaborators, retinoic-acid related orphan receptor-gamma (RORgamma) helps regulate lipid metabolism in the liver and serves as an important link between the circadian clock and the regulation of several lipid metabolic genes.

Previous studies showed that RORgamma exhibits a rhythmic pattern of expression that is controlled by the circadian clock, but its physiological function was poorly understood. Scientists fed RORgamma knockout mice and their control wild-type littermates a high fat diet or a normal diet, and analyzed gene expression and lipid levels in the liver at different intervals throughout the day. This study found that the rhythmic expression of RORgamma corresponded to the rhythmic expression of several genes important in lipid metabolism. This rhythmic regulation was greatly abolished in RORgamma knockout mice and resulted in significant changes in the level of several blood and hepatic lipids.

The investigators further demonstrated that some of these genes were indirectly regulated, while others were directly regulated by RORgamma through retinoic-acid orphan receptor response elements in their promoter regulatory region. The study reveals an important function for RORgamma in the rhythmic regulation of lipid metabolism and suggests a regulatory role for RORgamma in metabolic syndrome. **(SO)**

Citation: Takeda Y, Kang HS, Lih FB, Jiang H, Blaner WS, Jetten AM *(http://www.ncbi.nlm.nih.gov/pubmed/25143535)* . 2014. Retinoid acid-related orphan receptor gamma, RORgamma, participates in diurnal transcriptional regulation of lipid metabolic genes. Nucleic Acids Res 42(16):10448-10459.

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