Intramural papers of the month

By Heather Franco, John House, Mallikarjuna Metukuri, and Bailey Schug

DNA methylation could predict breast cancer risk

NIEHS scientists have discovered DNA methylation in blood could prove to be an effective indicator of who will develop breast cancer. Using the NIEHS Sister Study, a nationwide cohort of women, ages 35-74, whose sister had breast cancer, researchers used DNA extracted from white blood cell samples and assessed methylation at 27,000 sites across the genome. The team also examined known risk factors for breast cancer and genotyped women for nine common polymorphisms associated with breast cancer risk.

Epigenetic modifications, including DNA methylation, are increasingly recognized as important determinants of gene transcription. The team found evidence that women who subsequently develop breast cancer have different blood methylation profiles than women who remain cancer free, and that these methylation differences are detectable months to years before the clinical diagnosis of breast cancer.

The scientists also found that epigenetic modifications were significantly more accurate in predicting who will develop breast cancer than the known risk factors and polymorphisms, although they caution their test is not yet accurate enough for clinical use. These findings hold promise for breast cancer detection and risk prediction through methylation profiling of blood. (**BS**)

Citation: Xu Z, Bolick SC, DeRoo LA, Weinberg CR, Sandler DP, Taylor JA. *(http://www.ncbi.nlm.nih.gov/pubmed/23578854)* 2013. Epigenome-wide association study of breast cancer using prospectively collected Sister Study samples. J Natl Cancer Inst 105(10):694-700.

The mechanism of action of a natural estrogenic compound

Researchers at NIEHS recently identified the mechanism of action for the natural estrogenic compound diarylheptanoid (D3). The work could have therapeutic implications for estrogen withdrawal symptoms in women.

D3s are abundant in spices and vegetables that possess estrogenic activity. In the present study, scientists used D3 isolated from the medicinal plant *Curcuma comosa*. They found that D3 activated an estrogen responsive element luciferase reporter through estrogen receptor alpha (ERalpha) in human cells, and mouse lacking ERalpha models. Their molecular modeling studies suggested that D3 could be accommodated in the ERalpha binding pocket. D3 also increased early-phase and late-phase estrogen-regulated gene responses in ovariectomized wild-type animals similar to 17beta-estradiol (E2). When administered together, D3 and E2 exhibited no additive or antagonistic effects.

The authors concluded that D3 acted as a weak agonist of ERalpha, without interfering with the effect of endogenous estrogens in the in vivo model. The finding may support the therapeutic use of this plant in ovarian cycling women. The authors also proposed that their three-dimensional molecular modeling studies may shed light on how other nonsteroidal endocrine-disrupting compounds exert estrogenic activity through ERalpha. **(MM)**

Citation: Winuthayanon W, Piyachaturawat P, Suksamrarn A, Burns KA, Arao Y, Hewitt SC, Pedersen LC, Korach KS. *(http://www.ncbi.nlm.nih.gov/pubmed/23552522)* 2013. The natural estrogenic compound diarylheptanoid (D3): in vitro mechanisms of action and in vivo uterine responses via estrogen receptor alpha. Environ Health Perspect 121(4):433-439.

Human mitochondrial DNA polymerase ineffectively repairs acrolein-induced adducts

Researchers from NIEHS, in collaboration with scientists from Oregon Health and Science University, report that acrolein–induced adducts to mitochondrial DNA are bypassed by human mitochondrial DNA polymerase gamma at low fidelity, causing errors in DNA synthesis. Since these errors have the possibility of becoming mutations that could then lead to human diseases, such as neurodegenerative disorders, the research has implications for public health.

Acrolein is a mutagenic aldehyde produced by biological processes and by combustion of organic materials, including tobacco

smoke. Acrolein reacts with bases on DNA to form adducts that block DNA synthesis. In the nucleus, DNA adducts are repaired by multiple translesion synthesis polymerases that don't exist in animal cell mitochondria.

The researchers utilized single nucleotide incorporation and primer extension analyses to assess if mitochondrial polymerase gamma was able to overcome acrolein-induced DNA adducts in mitochondrial DNA. They found adenosine adducts were correctly and efficiently repaired. However, repair of minor groove guanine adducts, although able to be bypassed by pol gamma, exhibited reduced efficiency and low fidelity with a preference for incorporation of opposite-adduct purines. **(JH)**

Citation: Kasiviswanathan R, Minko IG, Lloyd RS, Copeland WC. *(http://www.ncbi.nlm.nih.gov/pubmed/23543747)* 2013. Translesion synthesis past acrolein-derived DNA adducts by human mitochondrial DNA polymerase gamma. J Biol Chem; doi:10.1074/jbc.M113.458802 [Online 30 March 2013].

Coordinating DNA double-strand break repair at both ends

Utilizing a novel technology they developed, researchers in the NIEHS Chromosome Stability Group were, for the first time, able to directly observe an essential event in an evolutionarily conserved process of repair of DNA double-strand breaks (DSBs). They also established a key role for a clinically relevant protein complex in this initial step of DNA repair, providing insight into human health conditions.

Through 2-D pulsed-field gel electrophoretic mobility shift assay analysis of mutant yeast strains, lead researcher James Westmoreland demonstrated coincident resection at both ends of DSBs and showed that the process is coordinated by the MRX complex and Sae2 proteins. Unexpectedly, he found that the necessity of these proteins in coincident resection differed depending on the type of DSB with a stronger requirement at dirty breaks, which are more similar to those that occur naturally as a result of various environmental agents.

Not only does this study establish a new technique that can be adapted in future studies to other organisms, but it also demonstrates the importance of a specific protein complex in the repair of DNA breaks. Further, as the genes that encode these proteins are mutated in multiple diseases, these results have important implications for human health. **(HF)**

Citation: Westmoreland JW, Resnick MA. *(http://www.ncbi.nlm.nih.gov/pubmed/23555316)* Coincident resection at both ends of random, gamma-induced double-strand breaks requires MRX (MRN), Sae2 (Ctp1), and Mre11-nuclease. PLoS Genet 9(3):e1003420.

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