Extramural papers of the month

By Nancy Lamontagne

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Read the current Superfund Research Program Research Brief. New issues are published on the first Wednesday of each month.

DNA methylation in the human genome

DNA methylation regulates the expression of genes that guide development and define cell types. In mammals, 70 to 80 percent of all cytidine-phosphate-guanosine (CpG) dinucleotides are methylated, but NIEHS-supported researchers report evidence that only a fraction of these CpGs likely participate in genome regulation in a developmental context. The DNA methylation signatures, called differentially methylated regions, that the researchers identified, can be used to guide new, more effective approaches that examine only the most informative portion of CpGs.

The researchers systematically investigated the DNA methylation of 42 whole-genome bisulphite sequencing data sets across 30 diverse human cell and tissue types. For cells and tissue undergoing normal development, they observed dynamic regulation for only 21.8 percent of autosomal CpGs. Genome-wide association studies showed that differentially methylated regions often contained single nucleotide polymorphisms associated with cancer and Alzheimer's disease. The investigators used their set of differentially methylated regions to correctly identify an unknown tissue sample and to classify the types of cells present in a heterogeneous sample.

Citation: Ziller MJ, Gu H, Muller F, Donaghey J, Tsai LT, Kohlbacher O, De Jager PL, Rosen ED, Bennett DA, Bernstein BE, Gnirke A, Meissner A.
(http://www.ncbi.nlm.nih.gov/pubmed/23925113)
2013. Charting a dynamic DNA methylation landscape of the human genome. Nature 500(7463):477-481.

Genome-wide sequencing links aristolochic acid to cancer

NIEHS grantees used genome-wide sequencing to identify a mutational signature of aristolochic acid that can link a person's cancer with exposure to the environmental mutagen. The research shows that genome-wide sequencing can be used to detect whether a person was exposed to carcinogens.

Aristolochic acid is found in *Aristolochia* plants, which have been used for medicinal purposes for more than 2,000 years. Recently, scientists discovered that consumption of the plants is associated with urothelial carcinoma of the upper urinary tract (UTUC). To determine the molecular signature of aristolochic acid in UTUC DNA, the researchers conducted exome sequencing of tumors from 19 individuals with documented exposure to aristolochic acid, and seven patients with no known exposure.

The analysis revealed an average of 753 mutations in each tumor in the aristolochic acid group, with 72 percent of the mutations being A:T-to-T:A transversions. For comparison, they found 91 mutations in tumors from the non-exposed group. The A:T-to-T:A mutational signature showed up frequently in oncogenes and tumor suppressor genes in UTUC tumors associated with aristolochic acid. The investigators also detected the aristolochic acid mutational signature in one patient's tumor from a UTUC cohort, without previous indication of aristolochic acid.

Citation: Hoang ML, Chen CH, Sidorenko VS, He J, Dickman KG, Yun BH, Moriya M, Niknafs N, Douville C, Karchin R, Turesky RJ, Pu YS, Vogelstein B, Papadopoulos N, Grollman AP, Kinzler KW, Rosenquist TA.
(http://www.ncbi.nlm.nih.gov/pubmed/23926200)
2013. Mutational signature of aristolochic acid exposure as revealed by whole-exome sequencing. Sci Transl Med 5(197):197ra102.

A better understanding of tissue growth and repair

NIEHS-supported work found that endothelial-derived epoxyeicosatrienoic acids (EETs) are critical in accelerating in vivo

tissue growth. The findings could lead to new therapies for improving tissue repair and wound healing in the liver, lungs, and kidneys.

EETs are lipid mediators that regulate pain, inflammation, angiogenesis, and vascular tone. To better understand the role of these lipid mediators in tissue regeneration, the researchers used genetic and pharmacological tools to control endogenous EET levels in seven animal models - liver regeneration, kidney compensatory growth, lung compensatory growth, angiogenesis Matrigel plug assay, corneal micropocket assay, wound healing, and neonatal retinal vessel formation.

The investigators found that EETs accelerated tissue growth and organ regeneration during liver regeneration, kidney compensatory growth, lung compensatory growth, wound healing, corneal neovascularization, and retinal vascularization. They observed the same outcomes when they administered synthetic EETs and saw delayed tissue regeneration when EET levels were genetically or pharmacologically lowered. Soluble epoxide hydrolase inhibitors, which elevate endogenous EET levels, promoted liver and lung regeneration.

Citation: Panigrahy D, Kalish BT, Huang S, Bielenberg DR, Le HD, Yang J, Edin ML, Lee CR, Benny O, Mudge DK, Butterfield CE, Mammoto A, Mammoto T, Inceoglu B, Jenkins RL, Simpson MA, Akino T, Lih FB, Tomer KB, Ingber DE, Hammock BD, Falck JR, Manthati VL, Kaipainen A, D'Amore PA, Puder M, Zeldin DC, Kieran MW.
(http://www.ncbi.nlm.nih.gov/pubmed/23898174)
2013. Epoxyeicosanoids promote organ and tissue regeneration. Proc Natl Acad Sci U S A 110(33):13528-13533.

BPA linked with obesity in children

NIEHS-funded research found that higher levels of urinary bisphenol A (BPA) were associated with an increased risk for obesity in children participating in the National Health and Nutrition Examination Survey (NHANES) from 2003 to 2010. The investigators did not find significant associations of BPA with any other chronic disease risk factors.

Children were classified into quartiles based on their urinary BPA levels (quartiles 1 to 4 = <1.3, 1.3-2.6, 2.6-4.9, and >4.9 nanograms/milliliter). The children with higher levels of BPA showed increased odds for obesity. Specifically, for quartiles 2 vs. 1, the odds ratio (OR) was 1.74, 95 percent confidence interval (CI) 1.17-2.60, P = .008; for quartiles 3 vs. 1, OR was 1.64, 95 percent and CI 1.09-2.47, P = .02; and for quartiles 4 vs. 1, OR was 2.01, 95 percent CI 1.36-2.98, P = .001. The same was true for having an abnormal waist circumference-to-height ratio: quartiles 2 vs. 1, OR 1.37, 95 percent CI 0.98-1.93, P = .07; quartiles 3 vs. 1, OR 1.41, 95 percent CI 1.07-1.87, P = .02; and quartiles 4 vs. 1, OR 1.55, 95 percent CI 1.12-2.15, P = .01. The researchers say that longitudinal analyses are needed to better understand the temporal relationships between BPA exposure and the development of obesity and chronic disease risk factors in children.

Citation: Eng DS, Lee JM, Gebremariam A, Meeker JD, Peterson K, Padmanabhan V.
(http://www.ncbi.nlm.nih.gov/pubmed/23958765)
2013. Bisphenol A and chronic disease risk factors in US children. Pediatrics 132(3):e637-e645.

Prenatal nitrate exposure and birth defects

With support from NIEHS, a large population-based, case-control study found that prenatal exposure to nitrate from drinking water was associated with certain birth defects. However, higher nitrate intake did not increase associations between prenatal nitrosatable drug use and birth defects, suggesting that endogenous formation of nitrosatable compounds might not be the underlying mechanism for the birth defects.

Researchers used data from the National Birth Defects Prevention Study to link addresses of 3,300 case mothers and 1,121 control mothers in Iowa and Texas to public water supplies and nitrate measurements. Mothers of babies with spina bifida were twice as likely [95 percent confidence interval (CI): 1.3, 3.2] to ingest 5 milligrams (mg) or more of nitrate daily from drinking water versus control mothers, who consumed less than 0.91 mg of nitrate daily. From one month before conception through the first trimester, mothers of babies with limb deficiency were 1.8 times more likely than control mothers to ingest 5.42 mg. or more of nitrate daily (95 percent CI: 1.1, 3.1). The results for cleft palate and cleft lip for this time period were similar, with mothers 1.9 (95 percent CI: 1.2, 3.1) and 1.8 (95 percent CI: 1.1, 3.1) times more likely to ingest the high levels of nitrate, respectively.

The researchers concluded that future studies of birth defects could focus on prenatal exposure to mixtures of contaminants in drinking water, since nitrate contamination occurs with other water contaminants.

Citation: Brender JD, Weyer PJ, Romitti PA, Mohanty BP, Shinde MU, Vuong AM, Sharkey JR, Dwivedi D, Horel SA, Kantamneni J, Huber JC, Zheng Q, Werler MM, Kelley KE, Griesenbeck JS, Zhan FB, Langlois PH, Suarez L, Canfield MA.
(http://www.ncbi.nlm.nih.gov/pubmed/23771435)
2013. Prenatal nitrate intake from drinking water and selected birth defects in offspring of participants in the National Birth Defects Prevention Study. Environ Health Perspect 121(9):1083-1089.

Mechanism for amyloid-beta accumulation in Alzheimer's disease

An NIEHS grantee and colleagues report that low levels of copper can accumulate in the brain, leading to amyloid-beta plaques that are the hallmark of Alzheimer's disease. Researchers determined the molecular mechanisms by which copper accelerates Alzheimer's disease - information that could be useful for developing preventive and therapeutic approaches to control neurotoxic amyloid-beta levels in the aging brain.

Normal mice, a mouse model of Alzheimer's disease, and human cells were used to study copper-induced amyloid-beta accumulation in the brain. In normal aging mice, they found that copper accumulated in brain capillaries, and the accumulation was associated with a decrease in the amyloid-beta transporter known as low-density lipoprotein receptor-related protein 1 (LRP1), which normally removes amyloid-beta from the brain. The copper accumulation was also linked to higher brain amyloid-beta levels. In human brain endothelial cells, normal levels of copper caused LRP1 down-regulation. In the mouse model of Alzheimer's disease, copper accumulated in brain capillaries and, unlike in control mice, in the parenchyma. In these mice, copper not only down-regulated LRP1 in brain capillaries but also increased amyloid-beta production and neuroinflammation.

Overall, this work demonstrated that copper's effect on amyloid-beta homeostasis in the brain depends on whether it accumulates in capillaries or in the parenchyma.

Citation: Singh I, Sagare AP, Coma M, Perlmutter D, Gelein R, Bell RD, Deane RJ, Zhong E, Parisi M, Ciszewski J, Kasper RT, Deane R.
(http://www.ncbi.nlm.nih.gov/pubmed/23959870)
2013. Low levels of copper disrupt brain amyloid-beta homeostasis by altering its production and clearance. Proc Natl Acad Sci U S A 110(36):14771-14776.

PBDE levels decrease in pregnant women after ban

A new study, supported in part by NIEHS, found that levels of polybrominated diphenyl ether (PBDE) flame retardants in pregnant women have dropped since being banned in California and phased out of U.S. production in 2003-2004. The PBDE levels fell more quickly than expected, given the environmental persistence of these compounds.

The researchers previously studied pregnant women seen at San Francisco General Hospital in 2008-2009, finding that these women had PBDE concentrations that were among the highest in pregnant women, worldwide. For the new study, the researchers recruited 36 demographically similar women from the same clinic in 2011-2012. The scientists found that PBDE serum levels in the pregnant women had dropped by two-thirds since the 2008-2009 measurements. Specifically, adjusted least-squares geometric mean concentrations of PBDEs decreased 65 percent (95 percent CI: 18, 130) from 90.0 nanograms per gram (ng/g) lipid (95 percent CI: 64.7, 125.2) to 54.6 ng/g lipid (95 percent CI: 39.2, 76.2) ($p = 0.004$). Also, PBDE metabolites, OH-PBDEs, decreased 6-fold ($p < 0.0001$), and BDE-47, -99, and -100 declined more than BDE-153.

The researchers said that PBDE exposures likely declined because of regulatory action, and that their findings can be used to evaluate public policies and inform future public health interventions.

Citation: Zota AR, Linderholm L, Park JS, Petreas M, Guo T, Privalsky ML, Zoeller RT, Woodruff TJ.
(http://www.ncbi.nlm.nih.gov/pubmed/24066858)
2013. Temporal comparison of PBDEs, OH-PBDEs, PCBs, and OH-PCBs in the serum of second trimester pregnant women recruited from San Francisco General Hospital, California. Environ Sci Technol 47(20):11776-11784.

DNA methylation in children

In one of the first studies to look at DNA methylation changes in children, NIEHS grantees report that DNA methylation changes detected at birth persist through early childhood. Epigenetic changes such as DNA methylation can occur because of environmental and age-related factors, and are thought to be involved in the development of disease.

The researchers used an immunoassay to measure global DNA methylation in 165 children at birth (cord blood) and again at age 3. On average, DNA methylation was significantly higher in the 3-year-old children than at birth ($p = 0.01$). However, for any individual child, the difference in methylation was less than would be expected by chance. They also found that the mother's BMI before pregnancy was negatively predictive of DNA methylation at birth and at age 3, even after the researchers accounted for the correlation between DNA methylation at the two time points.

Although the health impacts of small changes in DNA methylation aren't known, the researchers say their results imply that factors influencing DNA methylation during early childhood may have long-term effects. The findings also point to a need for more research on how factors, such as high BMI before pregnancy, could influence the trajectory of a child's health.

Citation: Herbstman JB, Wang S, Perera FP, Lederman SA, Vishnevetsky J, Rundle AG, Hoepner LA, Qu L, Tang D. *(http://www.ncbi.nlm.nih.gov/pubmed/24023780)*
2013. Predictors and consequences of global DNA methylation in cord blood and at three years. PLoS One 8(9):e72824.

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