Validity of animal models of developmental toxicity

Pascale Chavatte-Palmer

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Validity of animal models of developmental toxicity	O036
Pascale Chavatte-Palmer	
INRA	
Jouy en Josas, France	

Validity of animal models of developmental toxicity Pascale Chavatte-Palmer and Anne Couturier-Tarrade
UMR BDR, INRA, ENVA, Université Paris Saclay, 78350, Jouy en Josas, France
The risk assessment of chemicals is currently highly dependent on animals, especially for reproductive toxicity where numerous guidelines are proposed by OECD, some extending to the second generation. Developmental studies are usually required in a rodent (mostly rat) and a non-rodent (mostly rabbit). These tests are generally adequate to screen for a large number of chemicals. Nevertheless, some potential adverse effects are not currently investigated by default. The developmental origins of health and disease (DOHaD) demonstrate that early life exposure of both parents, before conception, during pregnancy and/or during neonatal development, may induce adverse outcomes in the offspring and in subsequent generations through epigenetic mechanisms. These effects may vary depending on metabolic and nutritional status of the parent, as well as their exposure to other chemical agents, abridged as the exposome, timing and way of exposure, as well as on offspring sex.
Moreover, classical dose-response curves are not obtained using endocrine disruptors and nanoparticular compounds, thus challenging classical toxicology protocols.
In this perspective, it is important to better determine long-term effects, mechanisms of actions and biomarkers such as epigenetic marks for DOHaD. In this context, the choice of the animal model should may not be limited to the above mentioned species as anatomical and physiological characteristics of the rat and rabbit models do not fully recapitulate human features. For example, gonadal meiosis takes place entirely during pregnancy in humans and in sheep, whereas meiosis continues after birth in rodents and even starts in the post-natal period in the rabbit. Thus, in the context of a suspicion of transgenerational effects of pregnancy exposure to toxicants, due to epigenetic modifications, a sheep model would be more appropriate.
Based on examples from our own work and that of others, the presentation will illustrate how DOHaD may induce changes in paradigm in developmental toxicology testing and why animal testing may be refined and possibly reduced by the use of alternative mammalian models.

Epigenetic DNA Methylation-mediated Programmed Reduced Neural Stem Cell Proliferation and Differentiation in SGA Offspring	O037
Michael Ross	
UCLA School of Medicine	
Los Angeles, USA	

Mina Desai PhD, Guang Han MD and Michael G. Ross MD, MPH Dept. of Ob/Gyn, LABioMed at Harbor-UCLA Med Ctr, Torrance, CA
OBJECTIVE: Small-for-gestational age (SGA) human newborns have an increased risk of hyperphagia and obesity, as well as a spectrum of neurologic and neurobehavioral abnormalities. We have shown that the SGA hypothalamic (appetite regulatory site) neural stem cells (NSCs) exhibit reduced proliferation and neuronal differentiation. Furthermore, there is preferential differentiation of NSCs towards astrocyte versus neuronal lineage. DNA methylation (DNA methyltransferase; DNMT1) regulates neurogenesis by maintaining NSC proliferation and suppressing premature differentiation. Once differentiation ensues, DNMT1 preferentially and promotes astroglial and inhibits neuronal fate. We hypothesized that the programmed dysfunction of NSC proliferation and differentiation in SGA offspring is epigenetically mediated via DNMT1. METHODS: Control rat dams received ad libitum food, whereas study dams were 50% food-restricted from pregnancy day 10 to term to create SGA newborns. Primary hypothalamic NSCs from 1 day old SGA and Controls newborns were cultured in complete or differentiation media and transfected with nonspecific or DNMT1-specific siRNA (20 nM). At day 5 of siRNA transfection, NSC proliferation and protein expression of specific markers of NSC (nestin), neuroproliferative transcription factor (Hes1), neurons (Tuj1) and astrocytes (GFAP) were determined.
RESULTS: Under basal conditions, SGA NSCs exhibited decreased DNMT1 and reduced proliferation and neurogenesis, but increased GFAP, as compared to controls. DNMT1 siRNA markedly decreased protein expression of DNMT1, confirming silencing. In both SGA and controls in complete media, DNMT1 siRNA inhibited NPC proliferation (0.5-fold), consistent with reduced expression of nestin (0.5-fold) and Hes1 (0.4-fold). In differentiation media, DNMT1 siRNA decreased expression of Tuj1 (0.6-fold) but increased GFAP (1.4-fold).
CONCLUSION: In SGA newborns, impaired neurogenesis is epigenetically mediated via reduction in DNMT1 expression, via suppression of the neuroproliferative factor Hes1. Premature NSC differentiation to astrocytes limits neuronal differentiation. It is likely that the anatomic and functional maturation of regions beyond the hypothalamus (e.g., cerebral cortex, hippocampus) may be impacted, contributing to poor cognitive and neurobehavioral competency in SGA offspring.

Epigenetic abnormalities in children exposed to toxicants	O038
Andrea Baccarelli	
Columbia University Mailman School of Public Health	
New York, USA	

Prenatal exposure to environmental toxicants, even at low levels, has been associated with a range of adverse health outcomes that may manifest themselves later in life. Epigenetic programming of gene expression has been widely indicated as a biological process that may mediate the effects of metals on postnatal health outcomes. The wide availability of laboratory methods for epigenome-wide association studies (EWAS) now permits to conduct agnostic screens of the influences of environmental exposures on the human epigenome. In particular, microarrays for DNA methylation-wide analysis are particularly cost effective and have been largely used in human cohorts. However, human data correlating prenatal exposure to metals with differences in DNA methylation in the child are still sparse. In this presentation, we will report novel findings from two EWAS data analyses conducted in the longitudinal pre-birth Project Viva cohort. We generated epigenome-wide methylation data using the Illumina Infinium 450K Methylation BeadChip in cord blood as well as during early childhood (range: 2.9 to 4.9 years) and mid- childhood (range: 6.7 to 10.5 years). We conducted the first EWAS analysis to identify methylation sites associated with prenatal levels of mercury exposures. A second EWAS analysis searched for methylation sites associated with lead exposures. Our EWAS analyses identified multiple methylation sites. We conducted in-silico analysis to determine the association of methylation at these sites with gene expression, as well as of correlation of blood methylation with target tissues, such as for instance brain methylation. We examine neurocognitive outcomes
and their association with metal-related methylation sites. These studies provide an example of the use of EWAS analysis in human studies of metals and open the way to mechanistic studies to identify the specific role of DNA methylation in metal toxicity.