

The iCys[®] Imaging Cytometer in the Epigenetic Toolbox for Autism Research

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Dr. LaSalle is a Professor of Microbiology and Immunology with a cross appointment in the Rowe Program in Human Genetics. She serves on the editorial board of the journal Human Molecular Genetics, is a member of the NIH study section on Developmental Brain Disorders, and reviews for a number of national and international funding agencies and journals. The research focus in Dr. LaSalle's laboratory is on epigenetics of neurodevelopmental disorders, including autism, Rett syndrome, Angelman syndrome, and 15q proximal duplication syndrome. Dr. LaSalle's laboratory has developed multiple innovative approaches for epigenetic investigations, including the use of T cell cloning for separating X-inactivation and heterogeneous methylation patterns, the use of laser scanning cytometry for quantitating immunofluorescence on brain tissue and tissue microarrays, and the use of fluorescence in situ hybridization to investigate neuronal nuclear organization in human brain. Dr. LaSalle's laboratory has been successful in the use of genomic and epigenomic technologies to investigate the role of MeCP2 in the pathogenesis of Rett syndrome and autism spectrum disorders.

Abstract

Autism is an increasingly common disorder of complex etiology, affected by multiple genetic and environmental influences. Epigenetic mechanisms act at the interface of genetic and environmental risk factors in autism. Methylation of CpG dinucleotides and methyl-specific binding proteins are part of an epigenetic pathway essential for parental imprinting and chromatin dynamics during normal brain development. Autism has several phenotypic features in common with the neurodevelopmental disorders with altered epigenetic pathways. Rett syndrome (RTT) is an X-linked pervasive developmental disorder caused by mutations in MECP2, which encodes methyl-CpG-binding protein 2 (MeCP2).

Current molecular methods are often inadequate for investigating epigenetic changes of individual cells in complex tissue such as brain. Over the past decade, we have used laser scanning cytometry for detecting epigenetic changes in human and mouse brain samples relevant to autism and autism spectrum disorders by the quantitation of immunofluorescence on tissue microarrays. Applications have included investigating mosaic X chromosome inactivation patterns in females with Rett syndrome, showing reduced MeCP2 levels in the majority of idiopathic autism brain samples, and comparing wild-type and MeCP2-mutant mouse brain samples for DNA methylation, histone acetylation and MeCP2-target gene changes.

Using the CompuCyte iCys, we are currently testing the hypothesis that perinatal exposure to persistent organic polybrominated diphenyl ethers (PBDEs) may result in epigenetic changes in developing neurons that impact the development of social and cognitive behavior. A mouse model with a truncation of MeCP2 (MeCP2308) has described social behavioral defects and thus is a useful mouse model for testing hypotheses of gene x environment interactions affecting social behavior. The widespread use of PBDEs as commercial flame retardants over the past decade has raised concern about human exposure to this new pollutant and potential effects on the developing brain particularly in genetically susceptible individuals. MeCP2308/+ dams bred to wild-type C57Bl6J

males were exposed daily to BDE-47 (0, 0.03, or 0.1 mg/kg/day) for 10 weeks (4 weeks pre-mating, 3 weeks *in utero*, 3 weeks lactation). The 0.1 mg/kg/day BDE-47 exposure negatively impacted fertility and litter survival specifically in MeCP2-mutant but not wild-type C57Bl6/J mice, suggesting an increased genetic susceptibility of MeCP2-mutant mice to BDE-47 in reproductive success. Independent BDE-47 effects were limited to early pre-weaning developmental tests with significant effects on sensory neurodevelopment and ultrasonic vocalizations. In contrast, MeCP2 genotype effects were predominant in juvenile and adult tests showing significant defects in social behaviors, activity and spatial learning. Significant BDE-47/MeCP2 interaction effects were ameliorating for ultrasonic vocalizations and social interaction time, but compounding for adult spatial learning, specifically in the heterozygous females. Because of the specificity of interaction effects in females, X chromosome inactivation and other epigenetic mechanisms involving MeCP2 are further being investigated by immunofluorescence and laser scanning cytometry in female mosaic brain sections.