Molecular pathology selected abstracts

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Cocaine-induced changes in sperm linked to cocaine resistance in male rat offspring

July 2022—Although offspring share similar DNA, their physical and behavioral differences are multifactorial. One of those factors is epigenetics, the chemical and structural modifications of DNA by proteins and enzymes. Whereas the DNA sequence is relatively stable, epigenetic modifications are dynamic, as they are critical to controlling gene expression in response to cellular development and environment. Reproductive cells, or gametes, carry half the normal set of chromosomal DNA. Advances in molecular technologies demonstrate that this DNA is epigenetically modified to influence traits of future offspring, referred to as transgenerational epigenetic inheritance. Complex traits, such as obesity, metabolic syndrome, fertility, and behavior, have been associated with transgenerational epigenetic inheritance. The inheritance has been perceived to be solely via maternal lineage given ova are much larger than sperm, with a greater abundance of mitochondria, proteins, and cellular resources. However, evidence increasingly shows that paternal epigenetic modifications also influence offspring phenotypes. Using a rat model to study biological and behavioral changes due to cocaine self-administration, the authors of the study featured herein had previously shown that voluntary ingestion of cocaine by male rats reduced the voluntary cocaine intake of their male offspring, a behavior not seen in the offspring of male rats exposed to a saline control. In contrast, the female offspring of male rats that ingested cocaine showed no differences in the rate of cocaine acquisition or level of cocaine intake compared to the saline control rats. The reasons for the differences between genders is undetermined, though it is postulated that the hormonal influences of testosterone, estrogen, and progesterone and differences relating to the part of the brain involved in stress and addiction likely contribute to the observed phenotypes. Changes in brain protein expression in brain-derived neurotropic factor (BDNF), which blunts the effect of cocaine, were seen only in male rat offspring. Coincidentally, epigenetic changes in BDNF gene promoter were detected in the semen of paternal rats exposed to cocaine. In the current study, the authors evaluated the epigenetic and micro RNA (miRNA) profiles of semen for clues regarding how cocaine exposure in paternal rats influenced the behavior of their male offspring. They showed that cocaine administration changes DNA methylation patterns in semen but not miRNA-expression patterns. Surprisingly, only the *Cdkn1a* and *Sgce* genes showed gene promotor hypomethylated status (suggesting a gene is expressed) in the cocaine-exposed rats but not in the saline-exposed control rats. The authors investigated the *Cdkn1a* gene further. Male rats that self-administered cocaine showed an increase in brain expression of *Cdkn1a*, an observation also found in other studies of rodents exposed to cocaine. Interestingly, *Cdkn1a* gene expression was increased in specific areas of the brains of the cocaine-naive male progeny of male rats exposed to cocaine but not in the saline control progeny. Therefore, the genetic changes seen in the brains of cocaine-naive male progeny were similar to those seen in cocaine-exposed rats, suggesting transgenerational transfer of genetic changes secondary to environmental exposures. To determine if the effects of cocaine on sperm were temporary or permanent, the study was repeated with a delay in mating to allow for spermatogenesis to renew. The repeat study showed no difference in *Cdkn1a* expression in the brains of the offspring of cocaine-exposed male rats versus saline control rats. The delay in mating also had no influence on cocaine self-administration behaviors between the progeny of cocaine-exposed male rats and saline controls. These findings demonstrate that the effects of cocaine on sperm and male progeny are temporary and support the role of epigenetics in mediating this process. The authors performed additional studies to determine whether virally mediated changes in *Cdkn1a* expression levels affected cocaine self-administration behavior. The studies reinforced findings about the effect of cocaine-induced upregulation of *Cdkn1a* on behavior. The authors' findings show that a transgenerational effect of cocaine-induced upregulation of *Cdkn1a*, hypomethylation of the

Cdkn1a promoter in semen, and increased *Cdkn1a* expression in cocaine-naive male progeny of cocaine-exposed males are associated with reduced self-administration of cocaine. This study raises such questions as, What is the role of *Cdkn1a* in cocaine use? Can there be a therapeutic target? How does epigenetic information from sperm selectively increase the expression of *Cdkn1a* in one part of the brain versus another? Finding answers to these questions may promote understanding of the effects of transgenerational epigenetic inheritance on human health and behavior.

Swinford-Jackson SE, Fant B, Wimmer ME, et al. Cocaine-induced changes in sperm *Cdkn1a* methylation are associated with cocaine resistance in male offspring. *J Neurosci*. 2022;42(14):2905–2916.

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Insights about cancer mutational signatures: a study from the UK

Clinical genetic testing of a patient's tumor sample is designed to provide diagnostic, disease status, therapeutic, and prognostic information. However, tumor profiling in clinical diagnostics is frequently limited to gene-targeted analysis, which involves less than one percent of the human genome and does not reflect the thousands of mutations in cancer genomes. Alternatively, a complete mutational profile can provide a treasure trove of molecular information about key genes, regions of the genome involved, mechanisms at play, environmental and therapeutic effects, and subclone development. The authors performed a large study to classify cancers based on patterns of somatic mutations using whole genome sequencing. The United Kingdom-based study, which was built on prior landmark studies, identified novel mutation signatures and reassessed the classification of cancers based on the cellular biology driving cancer-associated mutations. More than 15,000 cancer samples were collected in the United Kingdom as part of a prospective study to evaluate whole genome mutational signatures of 19 common tumor types. The authors detected approximately 300 million single-base substitutions (SBS), 3 million double-base substitutions (DBS), 150 million insertions/deletions, and 2 million rearrangements. The single- and double-nucleotide variants were further classified into mutational signatures based on the type of mutation present—96 channels for SBS (that is, cytosine to thymine substitution) and 78 channels for DBS. The authors identified 82 SBS and 27 DBS high-quality signatures, confirming 42 and nine previously described SBS and DBS signatures, respectively, in the Catalogue of Somatic Mutations in Cancer and adding 40 SBS and 18 DBS novel signatures. In an elegant bioinformatic approach, they identified the most common mutational signatures per tumor type and then detected rare mutational signatures using the large database. Mutational signatures were more similar within cancer types than between cancer types, suggesting organ-specific mechanisms of tumorigenesis. The number of mutational signatures per organ type was limited to five to 10 signatures per tumor type and a median of five common signatures. Viewing cancers in relation to mutational signatures provides the medical and research communities with a new opportunity to re-evaluate tumorigenesis and cellular biology. Only a few mutational signatures are associated with well-known cellular mechanisms or environmental- or therapy-related exposures. The mutational signature SBS1 is characterized by C>T mutations at CpG, due to methylcytosine deamination, and seen in the majority of cancers tested. In contrast, SBS2 and SBS13, also characterized by C>T mutations, are associated with APOBEC-related deamination. DBS1 and DBS2 are associated with ultraviolet light exposure and smoking, respectively. DBS5 and SBS90 are associated with prior platinum and duocarmycin exposure, respectively. Some mutational signatures show organ specificity. For example, SBS101 is characterized by C>T variants in two-thirds of hematological malignancies; SBS120 is dominated by T>C mutations in the central nervous system; and SBS122 is characterized by T>C mutations in sarcomas. Some of the mutational signatures are associated with a known key genetic driver of the cancer. However, there is an undefined driver of tumorigenesis in a number of these signatures. Furthermore, the authors noted that the underlying biological processes contributing to many of the mutational signatures are unknown and require further research. Advances in molecular technologies and the results of this study of mutational signatures support the use of whole genome sequencing for cancer research and diagnostic use. The authors have created a computer algorithm called Signature Fit Multi-Step (FitMS), which helps scientists and clinicians identify mutational signatures in their own cancer samples. Mutational signature data can be viewed on the authors' website, https://signal.mutationalsignatures.com/explore/study/6.

Degasperi A, Zou X, Amarante TD, et al. Substitution mutational signatures in whole-genome–sequenced cancers in the UK population. *Science*. 2022;376(6591). doi:10.1126/science.abl9283

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