Intramural papers of the month

By Jacqueline de Marchena, Monica Frazier, Melissa Kerr, and Bailey Schug

APOBEC cytidine deaminases generate many mutations in human cancers

NIEHS researchers and colleagues at the Broad Institute of MIT and Harvard in Cambridge, Mass., report that a set of proteins, known to protect against retroviruses and retrotransposons, can cause mutations that are widespread in human cancers.

These mutations, which have a characteristic mutation signature, are produced by apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deaminases. Scientists from the team developed an analysis to detect and statistically evaluate the prevalence of APOBEC mutations in 2,680 tumor samples, collected from 14 types of cancer. Using this strategy, they discovered that APOBEC enzymes produce the majority of mutations in some bladder, cervical, breast, head and neck, and lung tumors.

These researchers also found APOBEC signature mutations specifically in genes that have been implicated in cancer development and progression, highlighting a potential link between APOBEC enzymes and carcinogenesis. Furthermore, the tumor samples demonstrated a direct correlation between the levels of APOBEC3B and APOBEC3A mRNA transcript and the prevalence of APOBEC-type mutations. However, it is likely that APOBEC expression acts in concert with other factors to drive APOBEC mutagenesis. The authors suggest that APOBEC mutations may also be facilitated by any factor that increases the prevalence of single-stranded DNA, including DNA damaging agents. **(JDM)**

Citation: Roberts SA, Lawrence MS, Klimczak LJ, Grimm SA, Fargo D, Stojanov P, Kiezun A, Kryukov GV, Carter SL, Saksena G, Harris S, Shah RR, Resnick MA, Getz G, Gordenin DA.
(http://www.ncbi.nlm.nih.gov/pubmed/23852170)
2013. An APOBEC cytidine deaminase mutagenesis pattern is widespread in human cancers. Nat Genet; doi:10.1038/ng.2702 [Online 14 July 2013]. Story

Observing a DNA polymerase using time-resolved crystallography

NIEHS scientists utilized a technique called time-resolved crystallography to examine how a model human DNA polymerase beta (pol beta) chooses a nucleotide during DNA synthesis. The researchers employed natural substrates and followed product formation in real time with 15 different crystal structures. They were able to observe molecular adjustments at the active sites that hasten correct nucleotide insertion and deter incorrect insertion.

The X-ray crystallography technique they used confirmed features of the computational results the researchers had generated earlier, but also revealed new information. They found that pol beta changes its shape, depending on whether it incorporates a complementary base pair or correct nucleotide. This action permitted researchers to isolate and characterize intermediate structures during nucleotide insertion, helping assess and identify fidelity checkpoints at a structural level.

The study also found that pol beta forms a third metal binding site during correct, but not incorrect, nucleotide insertion, and pyrophosphate more easily dissociates after incorrect nucleotide insertion. Prior to this evidence, researchers believed that only two metal ion binding sites were used by all polymerases in their mechanism of action and pyrophosphate was released instantly. The researchers hope this information will lead to a better understanding of the potential causes of disease. **(BS)**

Citation: Freudenthal BD, Beard WA, Shock DD, Wilson SH.
(http://www.ncbi.nlm.nih.gov/pubmed/23827680)
2013. Observing a DNA polymerase choose right from wrong. Cell 154(1):157-168. Story

How specific stressors alter p53 binding and transactivation

Scientists from NIEHS determined that a variety of stresses can change how the transcription factor p53, a well-known tumor suppressor, binds to DNA across the genome in human cancer cells. The team also discovered 149 new genes targeted by the p53 transcription factor, including several that are important in tumor suppression. The findings may help researchers develop

better cancer treatment therapies.

In this study, the authors investigated the differences in p53 binding patterns and transcriptional responses, after exposing osteosarcoma cells to the DNA damaging agent Doxorubicin and the p53 stabilizer Nutlin-3. Interestingly, they found their DMSO vehicle treatment also induced a large degree of DNA binding by p53, but this binding was not associated with the transactivation of genes, as was observed for Doxorubicin and Nutlin. Both treatments resulted in stress-specific transcriptional responses.

In addition to discovering that just half the consensus-binding motif was sufficient *in vivo* for binding, they found many new putative p53 target genes that are associated with a milieu of critical processes, such as DNA repair and immune responses. These findings will be extremely useful to the future identification of novel therapeutic targets in cancer treatment. **(MF)**

Citation: Menendez D, Nguyen TA, Freudenberg JM, Mathew VJ, Anderson CW, Jothi R, Resnick MA. *(http://www.ncbi.nlm.nih.gov/pubmed/23775793)*
2013. Diverse stresses dramatically alter genome-wide p53 binding and transactivation landscape in human cancer cells. Nucleic Acids Res; doi:10.1093/nar/gkt504 [Online 17 June 2013].

Early mouse development influences norepinephrine neuron diversity

Applying a technique known as intersectional genetic fate mapping to the developing mouse brain, NIEHS researchers characterized neurons that produce and release norepinephrine (NE), a hormone and neurotransmitter, and, for the first time, developed a map of their communication pathways. Since NE neurons are involved in several physiological processes, such as food intake and sleep, and are lost in Parkinson's and Alzheimer's diseases, understanding NE neurons will lead to improvements in human health.

The research team labeled NE neurons in the fetal mouse and followed their maturation. The scientists found that NE neurons, derived from a specific rhombomere, or segment in the developing hindbrain, shared common features in the adult brain. Team members used this information to create a novel system for grouping NE neurons.

The group also determined that these various subgroups of NE neurons were talking to many different parts of the adult mouse brain, including the amygdala, hippocampus, hypothalamus, and many more. Prior to this study, neuroscientists believed only one group of NE neurons communicated with the cerebral cortex. The results will provide valuable groundwork for the study of particular NE neurons and the impact diseases or environmental exposures have on them. **(MK)**

Citation: Robertson SD, Plummer NW, de Marchena J, Jensen P. *(http://www.ncbi.nlm.nih.gov/pubmed/23852112)*
2013. Developmental origins of central norepinephrine neuron diversity. Nat Neurosci 16(8):1016-1023. Story

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