This Month in Genetics

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The Eyes Have It

Each year when I teach my genetics students about Knudsen's two-hit hypothesis and retinoblastoma, I get the same question: when somebody inherits an *RB1* mutation, why do tumors mostly occur in the retina? This is exactly the question that Xu et al. explore in their recent paper. The answer appears to be that, although loss of Rb is the key initiating event in retinoblastoma, it is the particular cellular environment in which this occurs that makes the difference in tumor formation. Retinoblastoma cells express markers of cone precursors but not those of other retinal cell types, implicating the cones as being the cells in which retinoblastomas arise. What makes these cells vulnerable to transformation when Rb is lost? The particular signaling circuitry expressed in these cells, which naturally includes high levels of MDM2 and N-Myc, is key. These proteins promote proliferation and dampen the ARF-mediated apoptotic response that is induced by Rb loss, thereby supporting oncogenesis. Knock-down of expression of either protein decreases proliferation of retinoblastoma cell lines. Overall, this work highlights particular signaling pathways that appear to be central to the development of retinoblastomas specifically in cone cells. This leads me to the answer I have for my students this year: not only do you have to have those first two hits in *RB1* to get a tumor, but the hits also have to occur in the right cellular environment to foster its proliferation.

Xu et al. (2009). Retinoblastoma has properties of a cone precursor tumor and depends upon cone-specific MDM2 signaling. Cell 137, 1018–1031. 10.1016/j.cell.2009.03.051.

Immune Response to Gene Therapy: Beyond the Target Protein

In considering gene therapy for genetic diseases, one of the things that people have worried about is that the target protein will be recognized as foreign and eliminated from the body. In a recent clinical trial of gene therapy for hemophilia B that used an adeno-associated virus as the vector, this seemed to occur in one of the nine patients. In this patient, Factor IX (F9) levels returned to baseline at the same time that the patient's liver enzymes became elevated, suggesting that liver cells transduced with the vector were being cleared by cytotoxic T lymphocytes (CTLs). Although this immune response might have been directed at the vector itself, Li et al. believed the kinetics of liver cell damage and loss of F9 suggest the CTLs were directed against F9 epitopes, despite the fact that these CTLs were not found. They explored their ideas in mice expressing the human MHC I allele that was found in this patient. When a vector expressing F9 is delivered to these mice, CTLs are generated—not against the primary F9 open reading frame, but rather against a cryptic epitope derived from an alternative reading frame of F9. Why this cryptic epitope is expressed isn't yet clear—maybe a regulatory element got lost or added in the making of the cDNA cassette—but it does seem to direct CTLs to transduced hepatocytes and thus reduce the levels of F9 achieved in the mice. This work is a cautionary tale for researchers pursuing gene therapy; the good news is that F9 codon optimization that eliminated the cryptic epitope allowed them to avoid CTL-mediated elimination of transduced hepatocytes in their model.

Li et al. (2009). Cellular immune response to cryptic epitopes during therapeutic gene transfer. Proc. Natl. Acad. Sci. Published online June 16, 2009. 10.1073/pnas.0902269106.

Studying Human Evolution in Mice

Could a single gene be the key to human language? *FOXP2* has been implicated in language development in several different ways, including the identification of gene disruptions in people with speech-language impairment and the fact that positive selection appears to have led to the fixation of two amino acid substitutions in the human version of *FOXP2* after the human lineage separated from chimpanzees. To study the role of the human form of *FOXP2*, Enard et al. put a humanized version of the *Foxp2* gene into mice. Lo and behold, despite differing from their wild-type littermates by only two amino acids in this one gene, pups with the humanized *Foxp2* vocalize at significantly lower frequencies than their wild-type littermates. Extensive characterization of these mice also revealed that they exhibit decreased exploratory behavior and decreased dopamine concentrations in the brain. In the basal ganglia, they also have medium spiny neurons with longer dendrites and increased synaptic plasticity. Opposite effects are observed in mice that express lower-than-normal levels of *Foxp2,* suggesting that the effects of the

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humanized gene are not simply reducing gene function. Do the changes to the vocalization frequency in these mice result from the differences documented in the basal ganglia? Circuits in this region of the brain have been implicated in speech and language, but further work is needed for researchers to determine whether this is the case and to further explore whether these changes were at least partly responsible for the development of human language.

Enard et al. A humanized version of Foxp2 affects cortico-basal ganglia circuits in mice. Cell 137, 961–971. 10.1016/j.cell.2009.03.041.

Better Predictions of miRNA Targets

Although microRNAs (miRNAs) are clearly important for the regulation of gene expression, the fact that they interact with their targets at as few as six nucleotides means that it has been difficult to identify the true targets of a particular miRNA. Several predictive algorithms have been designed to pull out these targets, but their predictions don't always agree, and they are plagued by false positives. Chi et al. took an experimental approach to miRNA target prediction. They have been working on a high-throughput method called HITS-CLIP, in which RNAs that have been cross-linked to a protein are immunoprecipitated and sequenced. In mouse brain, they immunoprecipitated argonaute to identify the RNAs crosslinked to this protein, which is involved in miRNA-mediated gene regulation. Two pools of RNA are pulled down: one corresponding to miRNAs and one to the mRNA targets. They could then try to match up the Ago-mRNA footprint with the miRNA seed sequences to predict true miRNA binding sites. Compared to bioinformatic approaches, this experimental method has increased sensitivity and specificity for target prediction and should be useful as a general methodology for the identification of meaningful miRNA-mRNA interactions.

Chi et al. (2009). Argonaute HITS-CLIP decodes miRNA-mRNA interaction maps. Nature, published online June 17, 2009. 10/1038/nature08170.

Using Genetics in a Randomized Controlled Trial

Elevated lipoprotein(a) levels are associated with heart attack, but whether this circumstantial evidence means there is a causal association has been a topic of discussion. We don't yet have a way to modulate lipoprotein(a) levels, so we can't figure out the causality of association in a typical randomized controlled trial. Instead, Kamstrup et al. used Mendelian randomization to study lipoprotein(a)'s effect on risk of heart attack. The basic idea is this: because alleles are randomly assorted to offspring, you can set up a pseudo-randomized controlled trial based on genotypes. Any other confounders that you don't measure should also be randomly assorted to the groups, so in this way you should be able to study the effect of your trait of interest. Through this type of study design, Kamstrup et al. show that the kringle IV type 2 polymorphism (KIV-2) in the *LPA* gene, which is the known polymorphism that has the biggest effect on lipoprotein(a) levels, is associated with a risk of heart attack in three population-based studies from Denmark. In the same samples, they confirmed that lipoprotein(a) levels themselves are associated with a risk of heart attack and that adjustment for the KIV-2 genotype attenuates these risks. In the Copenhagen City Heart Study sample, a doubling of plasma lipoprotein(a) levels gives a hazard ratio for heart attack of 1.08; in comparison, a doubling of the genetically elevated lipoprotein(a) levels in Kamstrup et al.'s sample gives a hazard ratio of 1.22. The authors argue that this latter estimate might be more accurate because genetically based differences in lipoprotein(a) levels last a lifetime, whereas single measurements of lipoprotein(a) are a snapshot of the overall picture. Regardless of the exact risk, these results cement the causal role of lipoprotein(a) in the risk of heart attack. The next step is figuring out how to modify these risks.

Kamstrup et al. (2009). Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. JAMA 301, 2331–2339.

This Month in Our Sister Journals

Array-CGH for Diagnosis of Mitochondrial Disorders

Molecular diagnosis of mitochondrial disorders can be quite challenging. Not only can mutations to the mitochondrial DNA (mtDNA) itself be the problem, but they can also be found in nuclear genes that encode components of the mitochondria or that are responsible for synthesis of mtDNA. Chinault et al. have streamlined the process for analysis of mtDNA deletion and depletion through the development of a custom oligonucleotide array that includes coverage for the entire mitochondrial genome as well as ~150 relevant nuclear genes. A critical aspect of this type of array is that the reference sample must be matched for tissue and age because mtDNA copy number varies between tissues and with age. If proper attention is not paid to the reference, depletions of mtDNA can be missed. Not only can this array be used to detect

the size and location of a deletion in any of these regions, but it can also be used to estimate the percentage of heteroplasmy for a mtDNA deletion. This type of approach should reduce the number of steps it takes to accurately find and characterize deletions that cause mitochondrial disorders.

Chinault et al. (2009). Application of dual-genome oligonucleotide array-based comparative genomic hybridization to the molecular diagnosis of mitochondrial DNA deletion and depletion syndromes. Genetics in Medicine 11, 518–526.

Is Epigenetics to Blame for Missing Heritability?

Genome-wide association studies have given us insight into complex disease, but the risk variation that we have so far pulled out from these studies does not come anywhere near to explaining the full heritability of most of these traits. Some argue that rare genetic variation is going to be the next frontier for research in complex traits. Monty Slatkin decided to explore whether epigenetic changes could explain this problem of missing heritability. In the first step toward this goal, he developed a simple framework to model epigenetic inheritance. Because the rate of gain and loss of epigenetic changes is likely to be higher than that of mutations, close relatives are less likely to share these changes than is implied by their relatedness. This makes it unlikely that inherited epigenetic changes account for the missing heritability in complex disease, unless these changes are much more common than mutations or they have a much larger effect on disease risk. Before we can fully tease this apart, we need better estimates of the rate of epigenetic change and how long these changes persist.

Slatkin (2009). Epigenetic inheritance and the missing heritability problem. Genetics 182, 845–850. Published online May 4, 2009. 10.1534/genetics.109.102798.