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CAUSES OF CHILDHOOD CANCER NEWSLETTER

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To bee or not to bee a feast fit for a Queen!

As we reported previously [see **C3, Vol 14, No 3**], murine studies have demonstrated that nutritional manipulation during pregnancy can alter methylation status of certain genes in offspring. In the mouse model system we described in that earlier report, both coat color and a propensity to obesity in the offspring can be permanently altered depending on the amount of folate/choline provided to female mice during pregnancy [Waterland RA Jirtle RL; **Molec Cell Biol 2003; 23:5293-5300**]. The question arises as to whether this maternal nutritional manipulation during fetal development permanently affects key genetic pathways and cells related to disease, cancer, longevity, and even, perhaps, in the case of bees, "royalty status". Until now, the mechanism by which bee larvae fed 'royal jelly' became queens was unknown. Royal jelly is a protein-rich secretion collected from glands on the heads of worker bees. Although genetically identical at the DNA level, the workers and queens show quite dissimilar appearances and capabilities. The queen can live up to 5 years where the worker might only live for a few months. The queen also has fully developed ovaries, where the worker bee has only rudimentary ovaries. The queen also does nothing much but seek out drones for mating and laying eggs; the worker feeds her, keeps her cool or warm as needed, and removes her waste. The attendant workers also distribute a queen-secreted pheromone to other workers that inhibit them from starting new queen cells.

Here, **Kucharski R et al [Science 2008; 319:1827-1830]**, in a series of elegantly described experiments, show that royal-jelly influences alteration of a key methyltransferase gene (DNMT1) in bee larvae, which determines whether the larva becomes a queen or a female worker bee. Using RNA interference technology, they artificially silenced the DNMT1 gene (DNMT1 siRNA). The researchers then targeted methylation status of a key gene, Dynactin p62, which has been previously shown to be differentially methylated during development. They found that there is clear decrease in methylation of Dynactin p62 in larvae destined to become queens compared to those destined to become workers. Interestingly, the strongest silencing using Dnmt3 siRNA occurred during the L2 to L3 larval transition. Newly hatched larvae were not affected, while the treatment was lethal for embryos. Further, they evaluated the control and Dnmt3-silenced larvae (mimicking the royal jelly-fed larvae) using the honeybee genomic oligonucleotide microarray and discovered several

groups of differentially expressed genes responsible for lipid transport, protein turnover, etc. They conclude that this model system will help inform future epigenetic studies that seek to understand shifts in developmental fate.

COMMENT: These observations have profound implications for human developmental biology and, in turn, our understanding of the mechanisms by which cancer may develop. We must assume that similar scenarios can occur during human fetal development. Can gene expression be permanently modified by maternal diet or other pregnancy-related exposures? If so, what is the implication for the newborn and beyond? Importantly, the study of honeybees points to the critical period of the timing of exposure. The greatest influence occurred during a specific larval stage. Given the differences in organ system development in humans, we will have to consider the influence of exposure timing during gestation. This is indeed a fascinating time and we are excited to exploit such animal models (mouse, bees, zebrafish) in our quest to help understand how childhood cancer may develop.
 Julie A. Ross

Out of Africa... into cancer?

The Human Genome Project, completed in 2003, mapped the location of the 25,000 or so genes that define our species. Subsequent efforts, such as the International HapMap project, have focused on discovering the variation within genes that makes each person (barring identical twins) unique. Sorting through this deluge of information to produce useful knowledge about gene-disease associations will certainly take years. In the meantime, population geneticists using these data are turning out interesting observations by the week. A recent report that compared the quantity and type of variation in European Americans (EA) and African Americans (AA) is a good example [Lohmueller K et al **Nature Genetics 2008; 451: 994-998**].

The investigators used data from the Applera corporation's resequencing of ~10,000 genes among 20 EAs and 15 AAs. The dataset included 39,440 single nucleotide polymorphisms (SNPs) which met stringent quality controls, 18,457 of which resulted in a change of amino acid. The PolyPhen algorithm was used to predict the likely functional consequences of these non-synonymous SNPs. Polyphen compares human SNPs to those in homologous sequences in several other mammals to

calculate a “conservation score”, using the logic that an allele is more likely to be detrimental if it does not appear frequently in evolutionary history. SNPs were predicted to be “benign”, “possibly damaging”, or “probably damaging” based on the conservation score. For each SNP the newer “derived” allele was determined by comparison to the chimpanzee genome. Finally, SNPs were classified as “private” if they were exclusive to one population. The mean and standard deviation (SD) for homo- and heterozygosity of each SNP in EAs was compared to that in AAs via the Mann-Whitney U-test, with analyses repeated restricting to the various classes of SNPs defined above.

Individuals in this sample were heterozygous at a mean of 1,962.4 non-synonymous SNPs (+/- 275.1 SD). AAs were heterozygous for a significantly greater number of SNPs compared to EAs for both synonymous (mean 3158.9 vs. 2357.7; $p < 6.2 \times 10^{-10}$) and non-synonymous (mean 2265.0 vs. 1735.5; $p < 6.2 \times 10^{-10}$) variants. However, EAs were more often homozygous for the derived allele of both synonymous and non-synonymous SNPs ($p < 7 \times 10^{-10}$ for both). The pattern of proportionally greater heterozygosity among AAs and homozygosity among EAs was also true for possibly and probably damaging SNPs. For instance, EAs were homozygous for probably damaging alleles at a mean of 33.2 SNPs versus 26.3 among AAs ($p < 3 \times 10^{-6}$). As would be expected if variants were truly deleterious, and hence selected against, the mean frequency of probably damaging derived alleles (0.099 in AAs and 0.108 in EAs) was less than that for benign alleles (0.200 in AAs and 0.238 in EAs).

Computer simulations were conducted to determine if the patterns of variation seen in the empirical data could be explained by each population’s demographic history, with a steady, constant population expansion among AAs but a bottleneck followed by rapid population expansion among EAs. These models confirmed that non-synonymous SNPs can indeed become more common in the latter population because of the lack of time for purifying selection to have removed damaging mutations accumulated during expansion. Accordingly, a larger proportion of alleles private to EAs (15.9%) were probably damaging than those private to AAs (12.1%).

COMMENT: The incidence of childhood cancer overall, and of most individual types, is higher among white children compared to black children in the United States, sometimes dramatically so. For instance, the rate of acute lymphoblastic leukemia among white children is 1.6 times that of black children, while Ewing’s sarcoma is *six times* as frequent. While environmental exposures surely explain some of the disparity, the present study suggests that inherent susceptibility is also quite important. More generally, this research highlights the need to consider childhood cancer in the context of natural selection since, prior to the very recent successes in treatment, it was nearly always fatal. While a gene that increases the risk of cancer well after reproductive age may be evolutionarily neutral, the same cannot be said for a gene that raises the probability that a carrier will die young. Insight into the genetic basis of childhood cancer, among other diseases, will continue to mount as the falling cost of gene sequencing enables the study of the full human genome in ever larger samples of people. Logan G. Spector

Swimming against the (polluted) tide

The relation between air pollution and childhood cancer has

been examined in many studies with inconsistent findings. Linking air pollution to disease outcomes is challenging due to the difficulty of measuring individual-level exposures. Even more difficult is assessing the contribution of parental germline exposure to air pollution on disease in the offspring.

In this article, Yauk C et al [PNAS 2008; 105 (2):605-610] examined whether particulate air pollution could cause germline DNA damage in inbred mice. Mice were divided into two groups and housed in the vicinity of two steel mills and a main highway. One group was exposed to the ambient, polluted air while the other group was exposed to highly purified air from the same location. The investigators then compared levels of mutation at the expanded simple tandem repeat (ESTR) locus, bulky DNA adducts, DNA strand breaks, and whole genome methylation at 3, 10, and 16 weeks of exposure. These time points represent the stages of spermatogenesis. In the sperm of mice exposed to unfiltered air for 10 weeks followed by a 6 week laboratory rest, the frequency of ESTR mutations was 1.6 times (95% confidence interval:1.3-1.9) greater than mice exposed to clean air. Because DNA collected at 3 and 10 weeks showed no increase in mutation frequency, these results imply that the damage originated in spermatogonial stem cells. DNA strand breaks were also increased by 5-7 fold in mice exposed to the polluted air at 3 and 10 weeks but returned to normal after the 6 week laboratory rest, indicating that the breaks were repaired. Air pollution exposure was also associated with an increase in whole genome DNA methylation at 10 weeks in the sperm and remained increased even after the 6 week break in the lab. Finally, the researchers determined whether sperm were exposed to particulate air pollutants through measurement of bulky DNA adducts. Although they were able to detect adducts in the lungs, they were not found in sperm.

COMMENT: This is a well designed study that addresses the question of whether particulate air pollution can cause germline DNA changes in a controlled experimental setting. Although this study demonstrates that components of air pollution can cause both genetic and epigenetic changes to sperm DNA, several questions remain to be answered. Since bulky DNA adducts were not detected in the sperm, the mechanism by which DNA changes occur is unclear. Bulky DNA adducts are a measure of polycyclic aromatic hydrocarbon exposure which are known DNA mutagens. The authors propose that the germline DNA damage could have resulted through indirect mechanisms such as oxidative stress, which generates reactive oxygen species that can damage DNA. The epigenetic effects of air pollution are particularly intriguing given that aberrant DNA methylation is known to play a role in imprinting diseases and cancer. It will be important to determine which genes are susceptible to air pollution induced methylation changes, whether the methylation changes persist after fertilization, and if there are any associated phenotypic outcomes in the offspring. This study illustrates the importance of animal models for determining the potential effects of environmental exposures in humans. The increasing pressure on industry to reduce pollution through green technology will hopefully alleviate the problem for future generations. Kimberly J. Johnson