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meeting point

Environmental epigenomics and disease susceptibility

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The Keystone symposium on 'Environmental Epigenomics and Disease Susceptibility' was held in late March 2011 at the Grove Park Inn Resort in Asheville, North Carolina, USA. The meeting helped to define the developing field of 'environmental epigenetics' and the research presented established its role in disease aetiology and susceptibility.

This was the first Keystone symposium organized in the area of environmental epigenetics. The meeting brought together a broad group of scientists researching topics as diverse as epidemiology, toxicology, clinical medicine, molecular and cellular biology, and epigenetics. The meeting was organized by Randy Jirtle (Duke U., USA), Moshe Szyf (McGill U., Canada), and Frederick Tyson (NIEHS, National Institutes of Health, USA) and had an almost equal distribution of graduate students, postdoctoral fellows, new and established investigators. The size of the meeting and the diversity of its attendees was an indication that the field of environmental epigenetics is quickly becoming established.

As the name suggests, environmental epigenomics seeks to understand the influence of the environment on the epigenome and the combined effect of these factors on human health. They are linked by epidemiological and experimental data indicating that prenatal and early postnatal exposure to environmental factors result in permanent epigenetic modifications, which influence the likelihood of developing adult-onset diseases and neurodegenerative disorders. In the words of the organizing committee, the meeting sought to "provide evidence that environmental exposures during early development can alter the risk of developing medical conditions, such as asthma, autism, cancer, cardiovascular disease, diabetes, obesity, and schizophrenia later in life by modifying the epigenome."

The Keynote address was given by Eva Jablonka (Tel Aviv U., Israel), who focused on the history of epigenetics, starting with the work of Conrad Waddington. She emphasized the importance of epigenetic transgenerational inheritance by mentioning several examples of this phenomenon, and suggested that future research needs to incorporate epigenetics into the fields of population biology and evolution. An interesting discussion that followed this talk addressed the terminology and definitions associated with epigenetic inheritance, and suggested ways of qualifying different routes and levels of epigenetic transmission that might reduce confusion in scientific and public discourse on the topic.

When DNA methylation was discovered (Singer *et al*, 1977), the term 'epigenetic inheritance' was used to describe the replication of the epigenome—DNA methylation—upon cell proliferation and mitosis (Singer *et al*, 1977). Unfortunately, the definition of inheritance refers to generational transmission of information and not the mitotic stability of information. Therefore, it was suggested by some that the stability and replication of the epigenome on cell proliferation and mitosis be referred to as 'mitotic stability', while the generational transmission of epigenetic information be defined as 'epigenetic inheritance'.

Fetal origins of adult disease

Ezra Susser (Columbia U., USA) discussed the influence on brain development of early gestational exposure to famine in the Dutch 'hunger winter' of 1944–1945, which is linked to increased rates of schizophrenia in offspring. These results were corroborated by an independent research group that found increased rates of schizophrenia to be associated with early gestational exposure to famine in the Chinese famine of 1959–1960. A third collaborative study by both groups in another part of China found similar results. Data from the Dutch studies showing that early prenatal famine has epigenetic effects on the IGF2 imprinted site—as well as post-mortem studies of schizophrenia that have focused on this and closely related sites—suggest that epigenetics might have a role in the aetiology of this disease. Epigenetic data from the Chinese studies are being analysed and might shed further light on this question.

Marcus Pembrey (U. College London, England) presented human epidemiological studies from Sweden (Överkalix cohort). He reviewed the impact of changes in the food supply during childhood in males, on adult disease in their offspring and grandchildren. He also reviewed the effects of paternal childhood smoking on offspring in the Avon Longitudinal Study of Parents and Children (ALSPAC). Transgenerational increases in mortality rates, cardiovascular disease, diabetes and obesity were observed in these cohorts after exposure to these factors. Some of the transgenerational phenotypes are potentially associated with the direct exposure of the germline to the factor in question—namely those in offspring and grandchildren of exposed paternal grandmothers, but not in grandchildren of exposed paternal grandfathers. Analysis of the specific epigenetic alterations induced is needed.

Gudrun Moore (Institute of Child Health, UK) discussed the effects of imprinting abnormalities and birth weight on

disease susceptibility in later life. Claudine Junien (INRA, France) discussed epigenetic programming related to sexual dimorphism. An interesting poster and short presentation by Martha Susiarjo (U. Pennsylvania, USA) demonstrated the effects of fetal exposure to bisphenol A (BPA), a plastic compound, on imprinted gene DNA-methylation profiles. Previous studies have linked human disease to changes in methylation. Further studies are needed to functionally link environmentally induced modifications to disease.

Gene regulation

Several talks and posters presented data on the way in which epigenetic modifications can regulate gene expression. Ian Wood (U. Leeds, UK) discussed the combined interactions of DNA methylation and histone modifications in the regulation of gene expression, and Victor Lobanenkov (National Institutes of Health, USA) considered the way in which alterations in CTCF-binding and DNA methylation influence infertility, spermatogenesis and cancer. Taken together, studies in this area demonstrated that epigenetic factors have roles in regulating genome activity. The crucial element to consider for environmental epigenetics is the mitotic stability of the epigenetic modifications and their effects on genome activity. The stability and maintenance of the epigenome can explain how, long after an exposure is removed, the permanence of the epigenetic alteration can alter genome activity later in development.

In vitro fertilization and stem cells

Several groups have mapped the differences between epigenetic methylation in embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). James Thomson (U. Wisconsin, USA) has generated many of the initial ESC and iPSC lines and has compared their methylomes, in collaboration with Joe Ecker's group. Methylation patterns in human ESCs and iPSCs are generally similar, but there are differences between populations, a few of which are consistent across iPSC lines. ESCs have approximately 25% non-CpG methylations of CHH and CHG sites, but differentiated cells have negligible levels. One interesting class of differences between human iPSCs and ESCs is differential non-CpG methylation in a few regions near telomeres and centromeres—differences that correlate with differential gene expression. Thomson also discussed mutational accumulation during genetic manipulation and long-term culture of human iPSCs.

Louise Laurent (U. California San Diego and TSRI, La Jolla, USA) presented comparisons of the methylomes of human ESC and iPSC lines, and correlated gene expression with DNA-methylation levels. She discussed the pervasive variability in DNA methylation among human iPSC cultures. The classic view of high promoter DNA methylation and decreased gene expression therefore needs to be reconsidered, and caution should be used when making assumptions.

An interesting poster and short talk by Toshi Shioda (Massachusetts General Hospital, USA) examined abnormal biallelic DNA methylation associated with gene expression in the conversion of iPSCs to germ-line cells. The epigenome has a crucial role in the cell biology and development of stem cells, as well as in procedures such as *in vitro* fertilization and cloning.

An interesting presentation by Wolf Reik (Babraham Institute, UK) discussed epigenetic reprogramming during mammalian development. An exciting observation is that in the zygote after fertilization, 5-hydroxymethylcytosine (5hmC) seems to be associated with the paternal pronucleus, but not the maternal pronucleus. The enzyme involved in 5hmC generation is TET3, and knocking it down reduces 5hmC levels in the zygote and elevates levels of 5-methylcytosine (5mC). Study of 5hmC in ESCs by genome-scale sequencing found that it localizes to exons, some promoters and CpG islands. It was suggested that 5hmC is associated with active genes and might facilitate demethylation at crucial stages of development, such as in the early embryo after fertilization. This is an insightful hypothesis, which requires further investigation.

Postnatal programming in the brain

The role of epigenetics and the effect of environmental factors on brain development and function were discussed by several speakers. Moshe Szyf (McGill U., Canada) discussed the way in which maternal care in rats alters DNA methylation in the brain and programmes the maternal-care behaviour of the offspring in later life. The impact of child abuse in early life on brain DNA methylation and behaviour in humans was also discussed in regard to suicide patients. Another model reviewed was the use of artificial mothers with non-human primates, which is found to alter brain DNA methylation and behaviour.

David Sweatt (U. Alabama, USA) used epigenetic modulation—through both histone deacetylase inhibitors and DNA methyltransferase inhibitors—to alter the epigenome early in development to influence long-term memory. Many of the corresponding gene-expression changes were also identified. Therefore, early-life environmental factors such as behaviour or therapeutics can alter brain programming and influence brain development and behaviour.

Epigenetics and complex disease

The correlation between environmental epigenetics and complex disease was discussed with regard to both human and animal models. Frederica Perera (Columbia U., USA) presented an interesting molecular epidemiology study that monitored the exposure of a group in New York City to PAH and BPA, and the resulting disease phenotypes. Exposures were correlated to asthma, behaviour changes and IQ changes. As expected, global DNA methylation in umbilical-cord blood was associated with prenatal exposure to PAH; specific sites also showed alterations in DNA methylation. This cohort is being followed through adolescence.

Maria Berdasco (IDIBELL, Spain) showed that DNA-methylation changes in the genome are related to the type of tumour examined. Differential methylation regions might be used in future to diagnose tumours and make prognoses. Thus, the human studies presented at the meeting support the premise that environmental influences on the epigenome are linked to disease onset in later life.

An interesting study presented by Dana Dolinoy (U. Michigan, USA) used the Agouti-mouse model, which responds to environmental factors, to alter a hypervariable epi-allele to change the coat colour of the animal. Fetal BPA exposures were found to alter coat colour, indicating that the chemical affects the epigenome at several dose levels. The alterations in the Agouti locus correlated with exposure and adult disease phenotypes.

Cheryl Walker (MD Anderson Cancer Center, USA) presented data from a rat model with a tumour-suppressor-gene defect, which she used to investigate the way in which environmental agents influence uterine tumour development. Uterine tumours are hormone-dependent, suggesting that environmental agents that mimic hormones such as BPA, diethylstibesterol (DES) and genestein could influence their

development. Exposure of neonates to these endocrine disruptors reprogrammed oestrogen-responsive genes, making them more sensitive to hormones and thereby promoting tumour development. DES and genistein had distinct effects in this model, increasing tumour development, but BPA did not. At the molecular level, reprogrammed genes exhibited alterations in epigenetic DNA and histone methylation, and induction of these alterations could be linked to activation of non-genomic signalling by these environmental compounds. These models demonstrate that environmental exposure in early life can alter the epigenome to influence disease later in life.

Transgenerational inheritance

The ability of environmental factors to promote epigenetic-induced disease and phenotypes—not only in the individual exposed, but also in subsequent generations by transmission through the germline—is important for biology and medicine. Emma Whitelaw (Queensland Institute of Medical Research, Australia) discussed the ability of the Agouti-mouse model to transmit epigenetic-induced coat-colour change transgenerationally through the female, but not the male germline. The model was also used to examine the effects of fetal alcohol exposure on coat-colour change and the promotion of abnormal phenotypes such as cranial facial development. The transgenerational transmission of this effect of alcohol has not been examined.

The ability of environmental compounds and toxicants to promote epigenetic transgenerational inheritance was discussed by Michael Skinner (Washington State U., USA). He presented data on the ability of the fungicide vinclozolin to promote permanent, epigenetic DNA-methylation changes in the sperm of both rat and mouse models, leading to several transgenerational adult-onset disease states. The actions of four compounds and mixtures of various toxicants were also found to promote epigenetic transgenerational adult-onset disease, as well as epigenomic changes in the sperm. An interesting poster and short talk were presented by Jennifer Wolstenholme (U. Virginia, USA), who discussed how gestational exposure to BPA promotes F1–F4 generation effects on social behaviours and corresponding *AVP* gene DNA-methylation changes.

Douglas Ruden (Wayne State U., USA) discussed a non-mammalian model of epigenetic transgenerational inheritance. He found that the exposure of an F0 generation of *Drosophila* to an HSP90 inhibitor promoted abnormal head structures for 13 generations. This was compared with the experiment by Conrad Waddington in the 1940s (Waddington, 1942), in which temperature was found to promote an abnormal wing phenotype for 16 generations. The hypothesis is that chromatin structure and non-coding RNA are involved at imprinting control-like regions.

One discussion that arose during these sessions was about the use of the term 'transgenerational'. If an individual or its original germline is exposed directly to an environmental factor, this cannot be considered a transgenerational phenotype. For example, the exposure of a gestating female to an environmental factor exposes the F0 generation (the mother), the F1 fetus and the germ line (within the fetus) that will later generate the F2 generation. Although F0, F1 and F2 generations can be affected, this is a multi-generational exposure, not involving transgenerational phenotypes. In this situation, an F3 generation would be required to investigate transgenerational phenotypes. If an adult female or male are exposed to an environmental factor, both the F0-generation adult and the F1-generation germline are directly exposed, such that an F2 generation would be needed for a transgenerational phenotype. The generation in which epigenetic transgenerational inheritance is observed must, by definition, not have been directly exposed at the level of either the germline or the organism.

Evolution and disease susceptibility

David Haig (Harvard U., USA) and Christopher Badcock (U. London, UK) discussed the role of mammalian evolution in disease aetiology. Genomic imprinting was the theme, and its influences on biology, social interactions and health were considered. Alterations in imprinting as a result of random mutation and in response to the environment can modulate physiological parameters such as reproduction, or promote disease conditions such as developmental and neurological defects. Randy Jirtle discussed the evolutionary stability and environmentally labile epigenome in this regard. The general concept is that evolutionary processes, developmental biology and epigenetic regulation of the genome will affect disease aetiology and biological processes. The analysis of imprinting provides a good model for the investgation of such epigenetic mechanisms and biological effects.

Conclusions

The meeting provided an in-depth review of the relatively new field of environmental epigenetics. The studies and observations discussed all support a crucial role for environmental epigenetics in disease aetiology and susceptibility. Many environmental factors—from nutrition to environmental toxicants—promote epigenetic changes during critical periods of development, to promote a permanent change in the epigenomes of cell populations, which later influence adult-onset disease and phenotypes in both present and future generations. Environmental epigenetics therefore provides a molecular mechanism for the fetal basis of adult-onset disease, and will be crucial to a full understanding of disease aetiology. It should be noted, however, that the impact of epigenetic mechanisms on basic biological processes, developmental biology, disease aetiology and evolutionary biology does not negate the importance of genetics in these processes. The interaction of epigenetic and genetic molecular events provides a more-powerful set of tools for the regulation of these processes.

In regard to environmental impacts on biology and disease, epigenetics provides an intermediate molecular mechanism to regulate genome activity, without compromising the nature of genome stability and DNA sequences. The incorporation of data and knowledge from environmental epigenetics into nearly all areas of biology is needed for a fuller understanding. There is a concomitant and urgent need to develop more-advanced and less-expensive epigenetic technology. It seems likely that progress in the field of environmental epigenetics will be rapid and will have an important impact on our understanding of disease and biology.

REFERENCES
Singer J *et al* (1977) *J Biol Chem* **252:** 5509–5513
Waddington CH (1942) *Endeavour* **1:** 18–20

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