Conference Scene

Epigenetics goes translational

Keystone Symposia on Molecular and Cellular Biology

Developmental Origins and Epigenesis in Human Health and Disease, 26–30 April 2010, Swissôtel, The Stamford, Singapore

Keystone Symposia held one of its five global conferences outside of North America in Singapore on 26–30 April 2010, entitled 'Developmental Origins and Epigenesis in Human Health and Disease'. This was organized by Judith Swain, Peter Gluckman, Michael Meaney and Anne Ferguson-Smith from the Singapore Institute for Clinical Sciences (SICS, Singapore), with 23 renowned international speakers and more than 100 delegates attending this symposium.

Developmental plasticity results from the interplay of fetal environment and genetic factors. These interactions play an important role in the susceptibility of an individual to developing chronic diseases and to the cognitive capacity of that individual. Evidence suggests that gene–environment interactions may result in epigenetic changes that are associated with susceptibility to chronic diseases, and may play a causative role. The specific nature of the procession through the signaling pathways that dictate these susceptibilities is still unknown. This symposium addressed the different experimental models of development, to understand the role of epigenetics in regulating important signaling pathways and to examine the relationship of the fetal environment to normal development and susceptibility to diseases.

The symposium started with a keynote address by Adrian Bird (University of Edinburgh, Edinburgh, UK) entitled: 'The CpG as a genomic signaling molecule'. MeCP2 is an abundant protein present in neurons that recruits corepressor complexes to create a repressive chromatin environment in a DNA methylation-dependent manner. Mutations in the *MeCP2* gene causes Rett's syndrome, but by reactivating the *MeCP2* gene, neurons can maintain homeostasis and reverse the symptoms of Rett's syndrome [1,2].

Anne Ferguson-Smith (SICS, Singapore, and University of Cambridge, Cambridge, UK) investigated the regulatory role of genomic imprinting in brain function and behavior. Delta-like homolog 1 (*Dlk1*), which is expressed specifically by the paternal chromosome, is a ligand that inhibits Notch signaling as well as Notch

independent signaling [3]. The dosage of *Dlk1* expression is important for the proper development in both heterozygote and homozygote *Dlk1* knockout mice that have reduced levels of neurogenesis in olfactory bulb neurons. They found that *Dlk1* is expressed in neural stem cells, and that neural stem cells from knockout mice were less capable of forming neurospheres *in vitro*. Importantly, Dr Ferguson Smith's laboratory demonstrated that *Dlk1* is not imprinted in astrocytes and stem cells in areas of the brain where neurogenesis occurs during adulthood. This supports the hypothesis that the control of gene dosage by the absence of imprinting is an important developmental factor.

Stephen Cohen (Temasek Life Sciences Laboratory, Singapore) created knockout *Drosophila* to systemically study miRNA genes and their function. *miR-8^{-/-}* *Drosophila* showed age-dependent neurodegeneration with increased apoptosis in the brain [4]. miR-8 targets four different sites located in the exons and the 3'-UTR of the Atrophin gene, a transcriptional corepressor, and limits its expression. Another notable miRNA is miR-14, the mutation of which is found in flies led to the development of obesity. These flies can be rescued by exogenous expression of miR-14, specifically in insulin-producing cells in the brain. On the contrary, overexpression of this miRNA in those cells causes a lean phenotype. One target inhibited by miR-14, in insulin-producing cells, is the transcription factor sugarbabe that controls glucose metabolism.

Emma Whitelaw (Queensland Institute of Medical Research, Queensland, Australia) is interested in epigenetic

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reprogramming between generations and the concept of totipotency that it is associated with [5]. Even within inbred littermates, they observed phenotypic variations due to epimutations, which are mitotically heritable. In this way, they studied the modification of frequency in methylation of the *Ay* allele as an example in a model of Fetal Alcohol Spectrum Disorder (FASD). Cross-fostering studies confirmed an effect mediated *in utero*. In order to identify genes involved in epigenetic reprogramming, her laboratory started a random mutagenesis screen using β -globulin-eGFP mice as a model. They identified 45 modifiers of murine metastable epiallele (MOMME) in mice. Focusing on the *Trim 28* gene, a transcription repressor, they observed that *Trim28*^{+/-}-bearing female mice started to become obese with age. Dr Whitelaw's work suggests that the 'developmental noise', the phenotype variations among genetically identical individuals in a controlled environment, is caused by epigenetic mechanisms, and leads to the hypothesis of a stochastic establishment of epigenetic mutations.

Caleb Finch (University of Southern California, CA, USA) presented his findings that support the hypothesis that an early exposure to infections decreases life expectancy. For example, the birth cohort that was prenatally exposed to the 1919 influenza pandemic shows a higher incidence of cardiovascular diseases [6]. Interestingly, there are also fewer graduates than other nonexposed cohorts, suggesting that early infection has also an effect on brain development. Finch also focused on pollution and cognitive decline, and demonstrated a positive correlation between airborne vehicular particles (mainly generated by airplanes) and carotid thickness, which in turn is associated with cognitive decline. As a conclusion, he predicted that high life expectancy would be reserved for a privileged group who are able to live in a protected environment, whereas for others, life expectancy might decrease.

Russell Fernald (Stanford University, CA, USA) reported on how social information regulates reproductive physiology and brain activity using the African cichlid fish, *Haplochromis burtoni*, as a

model [7]. He demonstrated that territorial fish, as compared with nonterritorial fish, have large functional gonads and large neurons with a dense network of dendritic connection in the preoptic area of the hypothalamus. A change in social status is sufficient to induce a change in gonad size and brain morphology. The methylation status of the gonadotropin-releasing hormone 1 gene is also tightly associated with the social status of male fish. These and other results presented provide us with a clue as to how environmental information derived from social interactions influences cellular and molecular processes in the brain, and how they shape behavior. Many of the aspects studied are conserved in humans.

The talk given by John Mattick (University of Queensland, Queensland, Australia) centered on the hypothesis that the emergence of noncoding RNA transcripts could be functionally linked to the development of complex organisms, including brain function, learning and memory. The data presented described the presence of multiple types of RNA for information storage and gene regulation, like 'transcription initiation RNAs', 'long noncoding RNAs' and 'nuclear splice RNAs' [8]. He also reported on the discovery of massive RNA editing in mammals, which is much more widespread than currently anticipated. Mattick suggested that RNA editing could serve as a mechanism for storing environmental information in the genome, on the level of the DNA sequence.

Peter Gluckman (SICS, Singapore and Liggins Institute, Auckland, New Zealand) presented a talk on the developmental contribution to the risk of chronic diseases [9]. Intrauterine growth retardation and physiological variations in the fetal experience can affect the epigenetic state of genes at birth like *RXR*, a gene that can predict phenotypic states in children aged 9 years from the Princess Anne Hospital nutrition study. Evidence suggests that fetal environmental cues are remembered in an organism, and potentially across generations.

Joseph Nadeau (Case Western Reserve University, OH, USA) talked about transgenerational epigenetic control of phenotypic variation in testicular cancer disease

risk [10]. The deficiency in Apobec1, a cytidine deaminase involved in RNA editing, shows a conventional inheritance in the paternal lineage but a reduced risk among sons of heterozygous mothers. This suggests that some transgenerational effects could be driven by mRNA granules transmitted by spermatozooids.

Michael Meaney (SICS, Singapore, and McGill University, Quebec, Canada) talked about how early experiences in life play a role in responses to health and disorders later in adulthood. His group studies how maternal care experiences can affect the offspring. He illustrated how tactile stimulation (high licking) contribute to the next three generations of high licking mothers as well as affecting the pathways associated with synaptic plasticity measured via long-term potentiation and depression. Furthermore, increased tactile stimulation also contributed to higher efficiency in spatial learning and memory [11–13].

Yi Eve Sun (UCLA, CA, USA) demonstrated how Dnmt3a is required for transcriptional activation of genes that promote neurogenesis through its occupancy and methylation on regions flanking proximal promoters of genes for neuronal differentiation. Dr Sun also showed that Dnmt3 antagonized the binding of polycomb repression complex 2 and emphasized the importance of Dnmt3a, as its deficiency impairs neuronal differentiation from neural stem/progenitor cells.

Zofia Zukowska (Georgetown University, NY, USA) is studying the molecular mechanisms behind a high-fat diet, stress and prenatal stress leading to adipogenesis and obesity. The group studies on the nonadrenergic cotransmitter, neuropeptide Y (NPY) and adult mice fed with a high-fat diet [14]. In these mice, stress activates both NPY and the Y2 receptor in abdominal fat. NPY is epigenetically regulated by DNA methylation at CpG in nerve growth factor responsive element and calmodulin responsive element regions. Stress exposure *in utero*, may involve the alteration methylation state alteration on NPY system in stem cells in neurovascular niche in fat pads, mostly affecting female offspring.

Judy Sng (SICS, Singapore) is interested in plasticity during brain development [UNPUBLISHED DATA]. The capacity for plasticity

in the adult is limited by the anatomical traces laid down during sensitive periods in early postnatal life. These sensitive periods are called ‘critical periods’. In the mouse visual cortex, the critical period has a clear onset and closure in dictating plasticity. Dr Sng reported that epigenetics, namely histone acetylation and DNA methylation, play a role in early brain development during the critical period window, and that perturbing the epigenome with a histone deacetylase inhibitor enables reactivation of adult visual cortical plasticity, measuring the ocular dominance shift by means of single-unit electrophysiology.

Jonathan Seckl (The Queen’s Medical Research Institute, UK) analyzes the effects of prenatal stress via fetal glucocorticoid excess persisting in the second generation. With maternal glucocorticoid therapy or ingestion of liquorice (which inhibits 11 β -hydroxysteroid dehydrogenase type 2, [11 β -HSD2]), his group found cognitive and behavioral impairments. He also studied two cohort groups: pregnant women who were exposed to the 9/11 atrocity during their third trimester and those who had experienced the Nazi Holocaust. It was found in both cases that such stress was transmitted to the offspring [15,16].

Michael Skinner (Washington State University, WA, USA) is studying the transgenerational effects of environmental toxicity when exposure takes place during embryonic gonadal sex determination (epigenetic programming and DNA remethylation) [17]. Using a genome-wide Methyl–DNA immunoprecipitation approach, he showed that vinclozolin, an endocrine disruptor, affects embryonic testis development, and subsequently, causes an increase in spermatogenic cell apoptosis in adult offspring for the next four generations.

Robert Waterland (Baylor College of Medicine, TX, USA) searches for metastable epialleles in humans [18]. From previous studies in mice, he found that maternal nutrition affects the establishment of epigenotype in early embryos, and is then maintained in differentiated lineages. Using peripheral blood leukocytes and hair follicle DNA, he found 40 candidate metastable epialleles. These studies will provide excellent candidate loci to explore epigenetic mechanisms in developmental origins of disease.

In short talks, Kian Peng Koh (Harvard, MA, USA) presented his work on Tet1/Cxxc6 and related enzymes as a new family of 5-methylcytosine hydroxylases in modulating pluripotency and cell lineage specification. Yijun Ruan (Genome Institute of Singapore, Singapore) talked about transcriptional regulation after estrogen induction using chromatin interaction analysis and paired-end tag sequencing ChIA-PET®. Assam El-Osta (Baker Heart Institute, Victoria, Australia) focused on hyperglycemia-inducing histone methylation and acetylation codes changes. Zhao Xu (Harvard) used epigenomics to understand adipogenesis. Corrado Spadafora (Istituto Superiore di Sanità, Rome, Italy) is studying the role of endogenous reverse transcriptase activity and retrotransposons in sperm cells and its potential to generate and propagate new

genetic information aside from that being carried in the chromosomes.

Overall, the audience of this Keystone Symposium was presented with an international showcase of rapid epigenetic studies moving towards being translational in several disease models, and we anticipate that new personalized therapies can emerge from these studies.

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