# Glucocorticoids and developmental 'programming' of brain and body

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Epidemiological evidence suggests that an adverse fetal environment permanently programmes physiology leading to increased risks of cardiometabolic, neuroendocrine and psychiatric disorders in adulthood [1]. We originally hypothesised that prenatal stress via fetal glucocorticoid excess might explain this link [2]. Indeed, in rodents, prenatal stress, glucocorticoid exposure or inhibition/knockout of 11ß-hydroxysteroid dehydrogenase type 2 (11□-HSD2), the feto-placental 'barrier' to maternal glucocorticoids, reduces birth weight and causes permanent hypertension, hyperglycaemia, increased hypothalamic-pituitary-adrenal (HPA) axis activity and anxiety-related behaviours in adult offspring [3]. The phenotype persists into a second generation and transmits via male and female lines. This implies epigenetic mediation, a mechanism emerging for at least HPA axis programming. This also appears of potential clinical relevance. Thus, in a singleton-bearing, non-human primate model, exposure to glucocorticoids in the second half of gestation programmes cardiometabolic, HPA and behavioural parameters in 1-year old offspring. In humans, placental 11□-HSD2 activity correlates directly with birth weight and inversely with infant blood pressure. Moreover, low birth weight babies have higher plasma cortisol levels throughout adult life, indicating HPA programming [4]. Indeed, maternal glucocorticoid therapy or ingestion of liquorice (which inhibits 11β-HSD) alters offspring cognition and affect [5]. Stress has similar effects since pregnant women exposed to the 9.11.2001 atrocity and who developed PTSD 'transmit' neuroendocrine changes to their one-year old offspring, but confined to third trimester exposure. Furthermore, exposure to the Nazi Holocaust exerted permanent effects upon glucocorticoid levels and steroid metabolism, effects dependent upon the age at exposure [6]. Second generation effects also occur. Overall, the data suggest that developmental exposure to excess glucocorticoids/stress programmes peripheral and CNS functions in adult life, predisposing to affective and other pathology, and may be transmitted into at least one subsequent generation.

# The genomics and epigenomics of smoking in pregnancy

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Over the past century, studies of development and reproductive biology have transcended our understandings of what constitutes heritability and the acquisition of phenotypic traits from one generation to the next. In recent years such investigations have evolved to focus on the role of *epigenetic modifications* to DNA and core histones in higher mammalian developmental processes. Two decades of research has led us to appreciate that one's epigenetic signatures are the net outcome of genotype, developmental lineage, and environmental exposures.

Our lab is dedicated to studying the effects of the *in utero* milieu on epigenetic changes in the human and non-human primate fetus in order to elucidate the mechanisms involved in aberrant regulation of fetal growth potential leading to risk of adult disease. In our human translational studies, we have focused our efforts on discerning the effects of a specific environmental exposure *in utero* (maternal tobacco use) on a single adverse fetal outcome (small for gestational age; SGA).

The metabolic pathways utilized by higher eukaryotic organisms to deal with potentially carcinogenic xenobiotic compounds from tobacco smoke have been well characterized. Carcinogenic compounds such as polycyclic aromatic hydrocarbons are metabolized sequentially in two-phases: in phase I *CYP1A1* catalyzes conversion into harmful hydrophilic DNA adducts, while in phase II *GSTT1* enables excretion via conjugation into polar electrophiles. In an effort to understand susceptibility to *in utero* tobacco exposure, we first characterized known metabolic functional polymorphisms and demonstrated that while deletion of fetal *GSTT1* significantly modified birth weight in smokers, no polymorphism fully accounted for fetal growth restriction. Since smoking upregulates *CYP1A1* expression, we hypothesized that non-allelic (epigenetic) dysregulation of placental *CYP1A1* expression via alterations in DNA methylation (meCpG) may further modify fetal growth. In the present manuscript, we compared placental expression of multiple CYP family members among gravidae, and observed significantly increased *CYP1A1* expression among smokers relative to controls (4.4-fold, $p<0.05$). To fully characterize *CYP1A1* meCpG status, bisulfite modification and sequencing of the entire proximal 1 kb promoter (containing 59 CpG sites) was performed. CpG sites immediately proximal to the 5'-XRE transcription factor binding element were significantly hypomethylated among smokers (55.6% vs 45.9% meCpG, $p=0.027$), a finding which uniquely correlated with placental gene expression ($r=0.737$, $p=0.007$). Thus *in utero* tobacco exposure significantly increases placental *CYP1A1* expression in association with differential methylation at a critical XRE element.

# Epigenetic plasticity in embryonic stem cells

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Normal development appears to take place through a unidirectional process characterized by a step-wise decrease in cell potency, and it is presumably this phenomenon that is mainly responsible for the difficulty in reprogramming of differentiated somatic cells in vivo. We showed that the histone methylase G9a is a master structural regulator that plays an important role in early development by targeting a wide network of embryonic genes for post-implantation repression. This silencing process includes key genes, such as Oct-3/4, Nanog and Dnmt3L that are intimately involved in maintaining the embryonic stem cell phenotype and for establishing maternal imprints in mammalian germ cells. As for the Oct-3/4 gene, it undergoes a novel multi-step program of inactivation that involves direct inhibition of transcription, heterochromatinization through the tri-methylation of H3K9 and subsequent DNA methylation that represents the major barrier to embryonic reprogramming.

microRNAs (miRs) are important regulators of post-transcriptional gene expression and are critical for stem cell maintenance and differentiation. miRs have also been shown to be involved in cancer pathogenesis as well. Most recently, we have shown that G9a also regulates the expression of a number of miRs both in undifferentiated embryonic stem (ES) cells and in early differentiated cells. Moreover, Gene Ontology analysis showed that these miRs are involved in several cellular and developmental functions and aberrantly regulated in various types of cancer. This work suggests a global mechanism for regulation of miRs during early differentiation of ES cells. This mechanism seems to imitate the silencing of many oncogenic miRs during early development whose de-repression in somatic cells may contribute to the development of cancer.

# DNA methylation and regulation of IGF1R expression in cancer progression

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The involvement of the insulin-like growth factors (IGF1, IGF2) in cancer biology has been the focus of extensive research. Epidemiological studies revealed that elevated serum IGF1 is associated with increased occurrence of various tumors, including breast, prostate, and colorectal cancer. The IGF1 receptor (IGF1R) is overexpressed in most malignant cells, where it displays a potent antiapoptotic activity. Regulation of IGF1R gene expression is an important mechanism that allows the cell to 'decide' whether to proliferate, go into arrest, or apoptose. Control of IGF1R gene expression at the transcriptional level has been identified as a key regulatory level. Using a novel DNA affinity chromatography protocol linked to mass spectroscopic proteomic analyses we identified a number of nuclear proteins with oncogenic or antioncogenic properties that regulate IGF1R gene transcription. Transcription factors with tumor suppressor activity, including p53, BRCA1, Von-Hippel Lindau (VHL), Wilms' tumor-1 (WT1), and others, were shown to negatively regulate IGF1R expression. The etiology of neoplasias associated with loss-of-function mutation of tumor suppressors is, in many cases, linked to the inability of mutant forms to suppress their molecular targets, including the IGF1R gene. On the other hand, gain-of-function mutations of oncogenes are usually associated with increased transactivation of the IGF1R promoter and/or augmented phosphorylation of its cytoplasmic domain and downstream signaling molecules. In addition, we evaluated the methylation status of the IGF1R and androgen receptor genes in a series of prostate cancer cell lines corresponding to early (benign) and advanced (metastatic) stages of the disease. Results of 5-Aza-2'-deoxycytidine experiments, methylation specific PCR, and sodium bisulfite-direct DNA sequencing revealed that the androgen receptor promoter is hypermethylated in metastatic, but not in benign, prostate cancer cell lines. On the other hand, no methylation was seen in the IGF1R promoter at any stage of the disease. In summary, interactions between transcriptional and epigenetic mechanisms may ultimately determine the level of expression of the IGF1R gene and, consequently, the proliferative status of the cell. Understanding the molecular basis of these interactions will be of significant value both in basic as well as translational terms.