Genomic imprinting as an adaptative model of developmental plasticity

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1. Developmental plasticity

Developmental plasticity can be defined as the ability of one genotype to produce a range of phenotypes in response to environmental conditions. Such plasticity can be manifest at the level of individual cells, an organ, or a whole organism. The totipotent zygote represents the pinnacle of cellular developmental plasticity and as the embryo develops, lineage restriction events reduce the developmental potential of subpopulations of cells such that cellular plasticity declines with developmental age. Epigenetic modifications, which can be defined as ‘the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states’ [1], play a key role in stabilising these lineage restriction events. Consequently, the reacquisition of developmental potential, as occurs naturally during germ cell development and fertilisation and artificially during cloning or during the generation of induced pluripotent stem cells (iPSCs), requires extensive epigenetic reprogramming. This includes the reprogramming of epigenetic marks at imprinted loci.

2. Genomic imprinting

Imprinted genes are a unique class of approximately 100 genes which are expressed predominantly from one chromosome in a parental-origin dependent manner (Fig. 1). Imprinted genes are not distributed uniformly through the genome, but are often found in clusters where the parental allele-specific pattern of gene expression is coordinately regulated by imprinting control regions (ICRs) through long-range cis-acting mechanisms. ICRs are characterised by differing epigenetic marks on the two parentally inherited chromosomes [2]. DNA methylation and the post-translational modification of core histones are important epigenetic modifications and these play key roles in imprinting control. To date, all ICRs identified are differentially DNA methylated regions (DMRs) on the two parental chromosomes. These differential methylation marks are acquired in the developing oocytes and sperm and, in normal circumstances, are heritably maintained after fertilization in the developing embryo and throughout life. Secondary or somatic DMRs, found at some imprinted promoter regions, acquire their parental-origin specific methylation post-fertilisation. This requires the gametic ICR and is thought to reinforce imprinted gene expression (Fig. 1, [3]). The role of histone modifications in imprinting control is less clear, however DMRs are characterised by the asymmetrical accumulation of different histone modifications on the two parental chromosomes and recently a requirement for histone demethylation in order to establish germline CpG methylation has been identified at some ICRs [4].

The existence of parental-origin specific DMRs necessitates a process of epigenetic reprogramming during gamete development such that germ cells exhibit the appropriate epigenetic marks at ICRs to ensure the successful development of future offspring (Fig. 1). This begins after the primordial germ cells (PGCs) have been specified at E7.5 and continues throughout the migration of the PGCs to the genital ridge. A second wave of demethylation occurs around E11.5 and includes the dramatic and rapid erasure of methylation at imprinted loci [5]. Dogma dictates that this demethylation is complete, and that there is no epigenetic
Inheritance through meiosis, however, some elements, such as intra-cisternal A particles, can partially escape this methylation reprogramming [6,7].

Imprinted genes have been proposed as key modulators of organismal developmental plasticity, but there is also evidence for their involvement in the plasticity of organs and single cells. There are two mechanisms through which the expression of an imprinted gene may be modulated: through a canonical, transcription factor driven mechanism (Fig. 2A), or through the modulation of imprinting itself (Fig. 2B). Imprinted genes are not universally mono-allelically expressed, rather the umbrella classification of “imprinted” conceals an extraordinary variety of temporal and tissue specificity of mono-allelic expression and, for some genes, inter-individual heterogeneity [8,9]. Our understanding of what initiates mono-allelic expression remains sketchy, although in some cases this coincides with differentiation events which restrict cellular developmental potential. Altered imprinted gene dosage through loss of imprinting, the activation of the normally silent allele, or the silencing of the normally active allele of an imprinted gene has been observed in various pathological states, however it remains unclear whether this is utilised as a mechanism of dosage control during normal development.

Fig. 1. Reprogramming of imprinting control regions during development. The existence of parental-origin specific DMRs necessitates a process of epigenetic reprogramming during gamete development such that germ cells exhibit the appropriate epigenetic marks at ICRs to ensure the successful development of future offspring. This begins after the primordial germ cells (PGCs) have been specified at E7.5 and continues throughout the migration of the PGCs to the genital ridge. A second wave of demethylation occurs around E11.5 and includes the dramatic and rapid erasure of methylation at imprinted loci (reviewed by Sasaki and Matsui [5]).

Fig. 2. Mechanisms of imprinted gene dosage regulation. Imprinted gene dosage may be modulated either through a canonical, transcription factor mediated mechanism where expression from the normally expressed allele is upregulated (A); or through the relaxation or loss of imprinting, where the normally silent allele is reactivated (B). Loss of imprinting may involve a single gene in an imprinted cluster (B) or the whole locus (C), with coordinate changes in expression of reciprocally imprinted genes. This is often associated with changes in epigenetic marks at imprinting control elements or secondary DMRs.
3. Developmental plasticity at the single-cell level

3.1. Imprinting dynamics in early development and in stem cells

Because the derivation and in vitro culture of embryonic stem (ES) cells are potential points of origin for epigenetic abnormalities, the epigenetic status of all stem cells and their derivatives must be established prior to their therapeutic use in humans. Recently, induced pluripotent stem cells (iPSCs) have been generated by the forced over-expression of defined sets of transcription factors in human somatic cells [10–12]. iPSCs hold great potential for the study of genetic diseases and to be a source of patient-specific stem cells for regenerative medicine therapies. Consequently the rigorous characterisation of these cells, including the epigenetic and expression status of imprinted loci, is of paramount importance. It remains unclear whether iPSCs are molecularly and functionally equivalent to blastocyst-derived ES cells. Recently a controversial study showed overall messenger RNA and microRNA expression patterns to be indistinguishable between murine iPSCs and ES cells with the exception of the aberrant silencing in some iPSC lines of the non-coding RNA transcripts of the imprinted Dlk1-Dio3 domain on chromosome 12qF1 [13]. This was associated with reduced contribution to chimaeras and a failure to produce viable all-iPSC derived mice, implying that expression of these imprinted non-coding RNAs is required for full developmental plasticity. While this study is compromised because restoration of expression and rescue of the phenotype was not conducted, it has rekindled the debate on imprinting status and its functional implications during early development and in stem cell models.

Evidence is accumulating for the relaxation of imprinting in the stem cell niche of some tissues. Our recent work on the role of the Dlk1-Dio3 locus in adult neurogenesis suggests that the selective modulation of imprinting is a normal mechanism of altering gene dosage and is associated with the control of developmental potential in the adult neurogenic niche [14]. We demonstrate that during early postnatal life, the normally silent maternal copy of Dlk1 is derepressed specifically in the multipotent stem cells of the neurogenic niche [14]. This is associated with the partial gain of methylation at the imprinting control region of this locus, the IgDMR. Interestingly, imprinting of Gtl2, a maternally expressed non-coding RNA in the same cluster is unaffected, and differential methylation of a secondary DMR at the Gtl2 promoter is maintained. Differentiation of adult neural stem cells both in vivo and in vitro is associated with the reacquisition of imprinting at Dlk1. These data force us to reconsider imprinting control mechanisms and the role of imprinting in developmental plasticity. However, it is currently unknown how many stem cell populations and imprinted genes behave in this way, and how mechanistically such dynamic imprinting modulation is achieved.

While the body of data on the tissue and temporal-specificity of imprinting at many loci is growing, the expression and imprinting status of imprinted genes during very early embryonic development remains largely uncharacterized. The emergence of monoallelic expression occurs at different developmental stages at different loci and also varies between different genes within a single imprinted locus. Data acquired from the study of undifferentiated ES cells derived from the pluripotent inner cell mass of the blastocyst have been used as an in vitro model of early development which complements in vivo data. This has revealed that at some loci the acquisition of mono-allelic expression occurs in tandem with differentiation or lineage restriction events. In undifferentiated ES cells equal expression has been shown from both Igf2r promoters. Differentiation is associated with the gain of imprinting at the Igf2r locus through the specific upregulation of expression from the maternally inherited allele [15]. In the early embryo, Igf2r is biallelically expressed from the 4 cell stage up to and including the blastocyst stage. Monoallelic expression is gained from E4.5-E6.5 and is dependent on the expression of the overlapping non-coding RNA Airm. Imprinting at the Kcnq1 cluster is also dependent on the expression of a non-coding RNA from the paternally inherited allele, Kcnq1ot1. In contrast to Airm, Kcnq1ot1 is paternally expressed in preimplantation embryos from the two-cell stage. Genes located close to Kcnq1ot1 are ubiquitously imprinted, and monoallelic expression is already detected in blastocysts and undifferentiated ES cells [16,17]. More distal genes are imprinted only in the extra-embryonic tissues and restriction of expression to one parentally-inherited allele coincides with trophoblast specification [17].

Together these data lead us to suggest that imprinting is a mechanism of dosage control which may, in some instances, be associated with the control of developmental potential. The careful study of the dynamics of imprinted gene expression at defined lineage restriction decisions in different cell populations and different developmental stages during in vivo development and in vitro differentiation and derivation are now required to test how widespread or rare such a strategy is.

3.2. Perturbation of imprinted gene dosage is associated with neoplastic transformation

Another interesting model in which to interrogate the role of imprinted genes in cellular plasticity is provided by cancer cells which are characterised by an abnormal gain in developmental potential. The importance of epimutation in cancer is increasingly being recognised. Indeed, some consider cancer to be as much an epigenetic disease as it is a genetic disease [18]. Many imprinted genes play roles in cellular growth and proliferation and consequently there may be selective pressure for their deregulation in cancer cells. Loss of imprinting (LOI) has been reported to be the most abundant alteration in some cancers and tends to be an early event in neoplastic transformation, demonstrating the importance of imprinted dosage in the maintenance of cellular and tissue identity [19–21]. Indeed, patients with congenital imprinting syndromes and deregulated imprinted gene dosage have an increased risk of cancer [22,23]. The gene encoding the insulin-like growth factor II (IGF2) and the H19 gene (a putative tumor suppressor gene) are imprinted in humans and expressed from the paternally inherited and maternally inherited allele respectively. Studies in solid tumours showed that the biallelic expression of IGF2 in gliomas and invasive breast cancers is associated with the aggressiveness of tumour growth [21]. There is evidence that LOI may predate and predispose to carcinogenesis, potentially by retarding cellular differentiation and derepressing developmental and proliferative potential (Fig. 3). Igf2 imprinting is lost in the colonic mucosa of 10% of the population and is associated with a personal and/or family history of colonic adenocarcinoma [24]. A murine Igf2 LOI model recapitulates the altered morphology of the normal colonic mucosa seen in patients with IGF2 LOI: an increased proportion of undifferentiated cells and expanded colonic crypts in the absence of proliferative changes [25]. This is associated with an increased incidence of colon cancer, strongly suggesting that LOI at the IGF2 locus promotes neoplastic transformation. While much literature documents LOI during neoplastic transformation, reports of transcription-factor mediated deregulation of imprinted gene expression in these processes is also growing. Imprinting is generally maintained at the DLK1-DIO3 locus in tumours, however, a variety of neuroendocrine and glial tumours are characterised by high levels of DLK1 expression, implicating dosage perturbation via a transcription-factor mediated mechanism [26]. A recent in vitro analysis found hypoxia-mediated...
DLK1 upregulation to be associated with increased “stemness”, tumourigenic potential and reduced differentiation [27], supporting the hypothesis that imprinted gene dosage may be related to tumorigenesis and malignant transformation (Fig. 3).

In each organ there exists a stem cell population which replenishes the cells of that organ through asymmetrical divisions, one cell remaining a stem cell while the other differentiates [28]. Several reports indicate that LOI in tissue-specific stem cells may cause the population to abnormally proliferate and expand [20,25,29]. Stem cells and cancer cells commonly share gene expression patterns, regulatory mechanisms, and signalling pathways. This has led to “the cancer stem cell hypothesis” which suggests that tumours arise from stem cell populations with dysregulated self-renewal caused by epigenetic and/or genetic initiating events, resulting in abnormal expansion and aberrant differentiation [25,30]. (Fig. 3). Furthermore, tumour cell heterogeneity has been proposed to be due in part to epigenetic variation and epigenetic plasticity in these progenitor cells [31]. As discussed above, there is evidence for the relaxation of imprinting in the stem cell niche of some tissues [14]. The mechanisms involved in regulating such selective relaxation of imprinting are almost entirely unknown, but are of potentially great importance to our understanding of how cellular developmental potential is controlled and the processes underlying neoplastic transformation.

4. Developmental plasticity of a whole organism

Organism developmental plasticity, the adaptive modification of developmental phenotype in response to environment, can result in astonishing phenotypic diversity. For example, polyphenism in invertebrates produces the colourfully different dry and wet season morphs of certain butterflies [32] or the sexual, asexual, wingless forms of the pea aphid [33]. In mammals there is increasing recognition of the power of the environment during prenatal development to shape adult growth, metabolism and behavioural phenotype. Indeed, studies on laboratory mice have shown that environmental influences can be a greater determinant of phenotype than genetic variation [34]. The study of genetically identical inbred mouse strains essentially eliminates inter-individual genetic variation, consequently any inter-individual phenotypic variation must stem from epigenetic differences.

As imprinted genes are crucial for reproductive and maternal behaviour, embryonic growth and the development and function of key metabolic axes (Fig. 4), they have been proposed as candidates to play a key role in mammalian developmental plasticity. It has also been hypothesised that, as the expression of imprinted genes is functionally monoallelic, exquisitely dosage sensitive and controlled by multiple layers of epigenetic regulation, imprinting and imprinted gene dosage may be more susceptible to environmental changes which impinge on the normal function of the cellular epigenetic apparatus [35]. However, we propose that the converse may instead be true: given the dependence of imprinted gene expression on epigenetic modifications, these may be more tightly safeguarded in the face of environmental perturbations during development and any mechanism which requires the action of the canonically repressed allele is likely to be highly regulated. Proper investigation of these hypotheses requires the analysis of how the expression of imprinted genes, as a class, responds to environmental challenge relative to the whole transcriptome and to other functionally related gene sets. In the absence of such analyses in the published literature we review the existing data on the stability of imprinted gene dosage and the epigenetic status of imprinted DMRs to environmental challenge during early life.

4.1. The role of imprinted genes in developmental plasticity in response to peri-conception environmental challenges

As the penultimate carbon donor to the methyltransferase enzymes is the essential amino acid methionine, diet may impinge on methyl-group availability for biological processes, including epigenetic modifications. It has been proposed that nutritional availability around conception may affect the post-fertilisation wave of epigenetic reprogramming [36,37]. Multiple studies of embryos fertilised and cultured in vitro have suggested that imprinting control elements may be more susceptible to the environment during this period than previously thought [38,39]. However, these studies are potentially confounded by the effects of superovulation, which has been shown to alter the epigenetic status of maternal ICRs [40].

There is some evidence of an association of peri-conception famine exposure with increased susceptibility to cardiovascular disease and earlier disease onset [41,42]. Exposure at this time point has been associated with subtle changes in methylation at three DMRs in different imprinted clusters in blood samples of affected versus unaffected sibs. However, the functional significance of this is unclear as leukocyte methylation is notoriously variable and the studies did not examine any associated expression changes, effects on imprinting, or attempt any correlation with known phenotypic outcomes [43,44]. In a rat model of peri-implantation low protein diet Kwong et al. [36,37] demonstrated a male-specific reduction in birth weight and development of hypertension at a young age, associated with a 30% reduction in male blastocyst H19 expression. While methylation at the H19 DMR was slightly altered [37], it did not correlate with the observed change in expression, indicating that it was not mechanistically responsible and, although not directly tested, imprinting
was likely to be intact. Furthermore, the phenotypic implications of a subtle reduction in $H19$ expression during early development are unknown. In summary, although in vitro studies provide some evidence that the epigenetic status of DMRs in the early embryo is labile and susceptible to culture conditions, there is currently little evidence for this from in vivo studies.

### 4.2. The role of placental imprinted gene expression in developmental plasticity

The placenta controls nutrient supply to the foetus, is the site of foeto-maternal interaction and is a highly active endocrine tissue, secreting factors which alter maternal metabolism and behaviour [45,46]. The placenta is also a highly plastic organ, responsive to foetal demand for resources [47]. Alterations in placental development can therefore have a dramatic effect on foetal growth; indeed, placental insufficiency is a leading cause of intra-uterine growth restriction in the developed world. Imprinted genes play key roles in placental growth, patterning and function and in the coordination of foetal resource demand and maternal supply, as exemplified by analysis of the $Peg3$ and $Igf2$ mutants [48–50]. Consequently several studies have sought to address whether deregulation of placental imprinted gene expression is associated with human developmental programming and intra-uterine growth restriction. The maternally expressed $Phlda2/Ipl$ acts to restrain placental growth while the paternally expressed $Mest$ promotes it [51,52]. Apostolidou et al. [53] screened 200 human placentas by qPCR for $PHLDA2$, $IGF2$, $IGF2R$ and $MEST$. Only $PHLDA2$ expression significantly correlated (negatively) with birth weight, but imprinting was not affected, implicating a transcription factor mediated mechanism. In contrast, McMinn et al. [54] assessed the transcriptome of a small sample of human IUGR and normal placentas and observed increased expression of $PHLDA2$ and decreased $MEST$. 

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**Fig. 4.** Imprinted genes are critical for the control of energy balance. The correct imprinted gene expression dosage is vital for energy homeostasis during both prenatal and postnatal life. Imprinted genes play critical roles in the control of foetal nutrient supply through effects on maternal metabolism and energy partitioning, placental development and function. There is also evidence that imprinted genes act coordinately in the foetus to regulate growth, thus altering foetal demand for maternal resources. Imprinted genes play key roles in the development of metabolic organs and modulate key adult metabolic pathways. Adapted from Charalambous et al. [66]
MEG3, GATM, GNAS and ZAC1 in IUGR placentas. They observed no methylation changes at the PHLD2A or MEST ICRs, nor were the spatial distribution of PHLD2A expression changed. Imprinted genes constituted 7% of their expression changes, a significantly higher proportion than would be expected, potentially implicating imprinted genes as a class as playing a key role in human IUGR. However, morphological adaptations occur in small placentas in an effort to sustain foetal growth [47] and thus the observed expression changes may be indirect, reflecting a secondary effect of the altered morphology.

In human studies much effort has focussed on establishing whether circulating foetal IGF2 levels correlate with foetal growth. However, the evidence is conflicting, as a variety of studies which have found that IGF2 levels in the placenta and/or cord blood correlate positively with birth weight [55–59], while others find no such relationship [60–64]. The discrepancies may partly be due to a failure to take into account the impact of changes in the levels of the circulating non-imprinted binding proteins which alter IGF2 bioavailability, IGFBPs. Serum level of several IGFBPs has been found to correlate with birth weight and may be modulated by in utero nutrition [57,58,61,65]. This relationship with proteins which modulate bioavailability makes Igf2 a particularly challenging model for assessing phenotypic plasticity and imprinted gene dosage and also suggests that the effective dosage of imprinted genes may be modulated post transcriptionally by non-imprinted pathways.

In summary, while there is some evidence of altered placental imprinted gene dosage in IUGR, there is no evidence that this involves changes in the epigenetic status of imprinted DMRs suggesting that transcription factor-mediated dosage modulation is responsible. Therefore, there is currently little evidence to suggest that placental imprinting is susceptible to environmental perturbation.

4.3. Imprinted genes and the postnatal sequelae of altered in utero development

The careful analysis of murine genetic models has demonstrated that imprinted genes play critical roles in the development of key metabolic organs with obvious consequences for postnatal metabolic phenotype. This includes neuroendocrine and endocrine organs involved in the control of homeostatic metabolic axes such as the brain, pituitary, adrenal and pancreas; as well as tissues critical for energy storage and utilisation such as muscle, white and brown adipose tissue and liver (Fig. 4) (reviewed in [66]). Perturbed development and postnatal function of these tissues is thought to contribute to the metabolic sequelae of developmental plasticity in response to in utero deprivation, but few studies have investigated whether altered somatic imprinted gene expression may be involved.

4.3.1. Imprinted genes and the pancreatic consequences of in utero growth restriction

Pancreatic sensitivity to blood levels of glucose, insulin, IGF1 and other hormones is critically important for metabolic health. The pancreas is a plastic organ, and early life events may play a role in determining the capacity for adult pancreatic plasticity. Several animal models have demonstrated altered pancreatic development following in utero deprivation [67–70]. A group of imprinted genes including Igf2, Rasgrf, Grb10, Neuronatin and Zac1 play key roles in pancreatic development and maturation and may be involved in the pathogenesis of these defects, although direct evidence of this remains scanty. Martin et al. [67] looked at the IGF axis and pancreatic function in rats which had been protein restricted in utero during the last week of gestation. These rats have a phenotype similar to that of local Igf2 overexpression [71] However, pancreatic Igf2 mRNA expression was reduced and there was no change in hepatic or serum levels of Igf2. Waterland and Garza [72] investigated the role of nutrition on pancreatic maturation by altering rat litter size during lactation. Both overnourished and undernourished animals had impaired pancreatic islet glucose-stimulated insulin secretion. Expression of Neuronatin was found to be significantly reduced in the overnourished individuals. Given the phenotypic similarities with an in vitro siRNA knockdown [73], reduced Neuronatin expression in this model may have contributed to the insulin secretory defects. However, interpretation of these data in the context of the whole pancreatic transcriptome is required to determine whether imprinted genes as a group are uniquely susceptible in the pancreas to environmental perturbation. Convincing evidence for the role of progressive reductions in pancreatic expression of the key transcription factors Hnf4α and Pdx1 following compromised early life conditions suggest that this may be unlikely [69,70].

4.3.2. Imprinted genes in brain development and the central control of metabolic axes

The brain is perhaps the most plastic organ of the body, capable of remarkable feats of learning and memory which involve rapid and widespread alterations of neuronal architecture and biochemistry. The majority of imprinted genes show high expression in the brain and many are imprinted only here [our observations, 74]. Furthermore, the human congenital imprinting syndromes, for example Prader–Willi syndrome (PWS) and Angelman syndrome (AS), are all characterised by neurological and behavioural impairments and learning difficulties, indicating the importance of imprinting in brain development and function [75]. Stress or deprivation in utero and negative experiences early in life have been associated in humans and animals with lasting changes in behaviour and emotionality and various psychiatric diseases [76]. Hotspots of imprinted gene expression are found in many areas critical for motivation, emotion and reward, such as the brainstem monoaminergic nuclei, the amygdala, nucleus accumbens and ventral tegmental area (our observations, [74,77]). While there has been much speculation on the possible role of imprinted genes in these areas and hence in psychiatric illness, direct evidence of this is sparse [78,79]. However Dlk1 and Grb10 have recently been shown to be involved in the development of the midbrain dopaminergic population [80], while loss of Magel2 is associated with defects in serotonergic signalling [81].

There is increasing evidence that developmental plasticity alters the central regulation of homeostatic axes such as those involved in control of blood volume, stress susceptibility and energy balance [82–84]. Many imprinted genes show high expression in key components of the hypothalamo–pituitary axis (our observations; [74,85]) and although genetic mouse models of altered dosage at the Dlk1-Dio3, Peg3 and Gnas loci show altered “set points” of metabolic axes there is, to our knowledge, currently no data linking changes in the early life environment with changes in the central nervous system expression of imprinted genes [86–89].

5. Concluding remarks

Dosage control at imprinted loci is essential for successful embryonic development. The temporal dynamics of acquisition of imprinted expression at certain loci coincide with cellular differentiation or lineage restriction events and the abnormal silencing of a cluster of imprinted non-coding RNAs has been associated with reduced developmental potential of iPSCs [13]. Conversely, recent data suggests that the highly selective and regulated relaxation
of imprinting is associated with cellular developmental potential in some stem cell populations [14]. Furthermore, loss of imprinting and altered imprint gene expression dosage has been associated with neoplastic transformation [21]. This leads us to suggest that imprinting may be associated with the control of cellular developmental plasticity. The investigation of the temporal dynamics of imprinting in vivo during early development and in further tissue-specific stem cell populations is required to determine the extent of the physiological role of imprint gene expression in cellular developmental plasticity.

It has been proposed that imprint genes may be more susceptible to dosage perturbation due to early life environmental challenges, and therefore that they may play a key role in the plastic developmental response of an organism to the early life environment. However, we propose that the opposite may be true, that imprinting genes may be protected from or may be less susceptible to such environmental perturbation. To properly test such hypotheses, the expression of imprint genes in the context of the whole transcriptome response to environmental challenge during early life must be assessed, and such data is currently lacking. Most studies have been hampered by low sample size, but there is emerging evidence that genes such as Phlda2 may be involved in altered placental development associated with intrauterine growth restriction. However, untangling cause and effect and in such a morphologically plastic tissue is complicated. Where there is some evidence of altered expression of imprint genes in developmental plasticity, this is generally not associated with substantial relaxation of imprinting, and does not consistently correlate with changes in DNA methylation, implicating transcription-factor mediated mechanisms, rather than loss of imprinting. Therefore, modulation of gene dosage through loss of imprinting, as a developmental mechanism, may be rare, and any mechanism which requires the action of the canonically repressed allele is likely to be highly regulated.

References

[1] Bird, A. (2007) Perceptions of epigenetics. Nature 447 (7143), 396–398.
[2] Edwards, C.A. and Ferguson-Smith, A.C. (2007) Mechanisms regulating imprinted genes in clusters. Curr. Opin. Cell Biol. 19 (3), 281–289.
[3] Edwards, C.A. et al. (2007) The evolution of imprinting: chromosomal mapping of orthologues of mammalian imprinted domains in monotreme and marsupial mammals. BMC Evol. Biol. 7, 157.
[4] Ciccone, D.N. et al. (2009) KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints. Nature 461 (7262), 415–418.
[5] Sasaki, H. and Matsui, Y. (2008) Epigenetic events in mammalian germ-cell development: reprogramming and beyond. Nat. Rev. Genet. 9 (2), 129–140.
[6] Lane, N. et al. (2003) Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. Genesis 35 (2), 88–93.
[7] Rakyan, V.K. et al. (2003) Transgenerational inheritance of epigenetic states at the murine Atrx/Fu allele occurs after maternal and paternal transmission. Proc. Natl. Acad. Sci. USA 100 (5), 2538–2543.
[8] DeChiara, T.M., Efstratiadis, A. and Robertson, E.J. (1990) A growth-deficiency gene product of the Dlk1-Dio1 region in the rat embryo-fetal axis is altered in response to periconceptual maternal low protein diet. Reproduction 132 (2), 265–277.
[9] D’Angelo, R.C. and Wachtmeister, C.A. (2010) Stem cells in normal development and cancer. Prog. Mol. Biol. Transl. Sci. 95, 113–158.
[10] Kooi, R.E. and Brakefield, P.M. (1999) The critical period for wing pattern induction in the polyphagous tropical butterfly Bicyclus anynana (Satyrinae). J. Insect Physiol. 45 (3), 201–212.
[11] Genotype sequence of the pea aphid Acyrthosiphon pisum. PLoS Biol. 8(2), e1000313.
[12] Francis, D.D. et al. (2003) Epigenetic sources of behavioral differences in mice. Nat. Neurosci. 6 (5), 445–446.
[13] Jirle, R.L. and Skinner, M.K. (2007) Environmental epigenomics and disease susceptibility. Nat. Rev. Genet. 8, 253–262.
[14] Kwong, W.Y. et al. (2000) Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. Development 127 (19), 4195–4202.
[15] Kwong, W.Y. et al. (2006) Generation of mouse induced pluripotent stem cells using overexpression of the IGF2 gene in the rat embryo-fetal axis is altered in response to periconceptual maternal low protein diet. Reproduction 132 (2), 265–277.
[16] Lewis, A. et al. (2006) Epigenetic dynamics of the Kcnq1 imprinted domain in the early embryo. Development 133 (21), 4203–4210.
[17] Rivera, R.M. et al. (2008) Et al., Manipulations of mouse embryos prior to implantation result in aberrant expression of imprinted genes on day 9.5 of development. Hum. Mol. Genet. 17 (1), 1–14.
[18] Isa, J.P. (2008) Cancer prevention: epigenetics steps up to the plate. Cancer Prev. Res. (Phil.) 1 (1), 6–7.
[19] Albrecht, S. et al. (1996) Variable imprinting of H19 and IGF2 in fetal cerebellum and medulloblastoma. J. Neuropathol. Exp. Neurol. 55 (12), 1270–1276.
[20] Yuan, E. et al. (2005) Genomic profiling maps loss of heterozygosity and defines the timing and stage dependence of epigenetic and genetic events in Wilms’ tumors. Mol. Cancer Res. 3 (9), 493–502.
[21] Jelicic, P. and Shaw, P. (2007) Loss of imprinting and cancer. J. Pathol. 211 (3), 261–268.
[22] Björnsson, H.T. et al. (2007) Epigenetic specificity of loss of imprinting of the IGFL2 gene in Wilms tumors. J. Natl. Cancer Inst. 99 (16), 1270–1273.
[23] Lin, D.H. and Maher, E.R. (2010) Genomic imprinting syndromes and cancer. Adv. Genet. 70, 145–175.
[24] Cui, H. et al. (2003) Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 299 (5613), 1753–1755.
[25] Sakatani, T. et al. (2005) Loss of imprinting of Igf2 alters intestinal maturation and tumorigenesis in mice. Science 307 (5717), 1976–1978.
[26] Yin, D. et al. (2006) DLK1: increased expression in gliomas and associated with oncogenic activities. Oncogene 25 (13), 1852–1861.
[27] Kim, Y., Lin, Q., Zelterman, D. and Yun, Z. (2009) Hypoxia-regulated delta-like 1 homologue enhances cancers cell stemness and tumorigenicity. Cancer Res. 69 (24), 9271–9280.
[28] Barker, N., Bartfeld, S. and Clevers, H. (2010) Tissue-resident adult stem cell populations of rapidly self-renewing organs. Cell Stem Cell 7 (6), 656–670.
[29] Caldecott, R. et al. (1999) Mosaic allele insular-like growth factor 2 expression patterns reveal a link between Wilms tumours and epigenetic heterogeneity. Cancer Res. 59 (16), 3889–3892.
[30] Feinberg, A.P., Ohlsson, R. and Henikoff, S. (2006) The epigenetic progenitor origin of human cancer. Nat. Rev. Genet. 7 (1), 21–33.
[31] Francis, D.D. et al. (2003) Epigenetic sources of behavioral differences in mice. Nat. Neurosci. 6 (5), 445–446.
[32] Jirtle, R.L. and Skinner, M.K. (2007) Environmental epigenomics and disease susceptibility. Nat. Rev. Genet. 8, 253–262.
Salas, M. et al. (2004) Placental growth retardation due to loss of imprinting of Phlda2. J. Hum. Genet. 121 (10), 1999–2010.

Lefebvre, L. et al. (1998) Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest. Nat. Genet. 20 (2), 163–169.

Apostolidou, S. et al. (2007) Elevated placental expression of the imprinted PHLDAla2 gene is associated with low birth weight. J. Mol. Med. 85 (4), 379–387.

McMinn, J. et al. (2006) Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. Placenta 27 (6–7), 540–549.

Akram, S.K. et al. (2008) Human placental IGF-I and IGF-II expression: correlating maternal and infant anthropometric variables and microintrauterine growth retardation at birth in the Pakistani population. Acta Paediatr. 97 (10), 1443–1448.

Leger, J. et al. (1996) Growth factors and intrauterine growth retardation. II. Serum growth hormone, insulin-like growth factor (IGF-I), and IGF-binding protein 3 levels in children with intrauterine growth retardation compared with normal control subjects: prospective study from birth to two years of age. Study Group of IUGR. Pediatr. Res. 40 (1), 101–107.

Giudice, L.C. et al. (1995) Insulin-like growth factors and their binding proteins. Rev. Endocr. Metab. 80 (5), 1548–1555.

Lo, Y.F. et al. (2002) Placental weight and birth characteristics of healthy singleton newborns. Acta Paediatr. Taiwan 43 (1), 21–25.

Ong, K. (2000) Et al., Size at birth and cord blood levels of insulin, insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein 1 (IGFBP-1), IGFBP-3, and the soluble IGF-II/mannose-6-phosphate receptor in term human infants. The ALSPEC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. J. Clin. Endocrinol. Metab. 85 (11), 4266–4269.

Hung, T.Y. et al. (2008) Relationship between umbilical cord blood insulin-like growth factors and anthropometry in term newborns. Acta Paediatr. Taiwan 49 (1), 19–23.

Osorio, M. et al. (1996) Insulin-like growth factors (IGFs) and IGF binding proteins-1, -2, and -3 in newborn serum: relationships to fetoplacental growth at term. Early Hum. Dev. 46 (1–2), 15–26.

Christou, H. et al. (2001) Cord blood leptin and insulin-like growth factor levels are independent predictors of fetal growth. J. Clin. Endocrinol. Metab. 86 (2), 935–938.

Reece, E.A. et al. (1994) The relation between human fetal growth and fetal blood levels of insulin-like growth factors I and II, their binding proteins, and receptors. Obstet. Gynecol. 84 (1), 88–95.

Wiznitzer, A. et al. (1998) Insulin-like growth factors, their binding proteins, and fetal macrosomia in offspring of nondiabetic pregnant women. Am. J. Perinatol. 15 (1), 23–28.

Street, M.E., Seghini, P., Fieni, S., Ziveri, M.A., Volta, C., Martorana, D., Viani, I., Gramellini, D. and Bernasconi, S. (2006) Changes in interleukin-6 and IGF system and their relationships in placenta and cord blood in newborns with fetal growth restriction compared with controls. Eur. J. Endocrinol. 155 (4), 567–574.

Charalambous, M., Da Rocha, S.T. and Ferguson-Smith, A.C. (2007) Genomic imprinting, growth control and the allocation of nutritional resources: consequences for postnatal life. Curr. Opin. Endocrinol. Diabetes Obes. 14 (1), 3–12.

Martin, M.A. et al. (2005) Protein-caloric food restriction affects insulin-like growth factor system in fetal Wistar rat. Endocrinology 146 (3), 1364–1371.

Jimenez-Chillaron, J.C. et al. (2005) Beta-cell secretory dysfunction in the pathogenesis of low birth weight–associated diabetes: a murine model. Diabetes 54 (3), 702–711.

Park, J.H. et al. (2008) Development of type 2 diabetes following intrauterine growth retardation in rats is associated with progressive epigenetic silencing of Pdx1. J. Clin. Invest. 118 (6), 2316–2324.

Sandovici, I. et al. (2011) Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the Hnf4a gene in rat pancreatic islets. Proc. Natl. Acad. Sci. USA 108 (13), 5449–5454.

Devedjian, J.C. et al. (2000) Transgenic mice overexpressing insulin-like growth factor-II in beta cells develop type 2 diabetes. J. Clin. Invest. 105 (6), 731–740.

Waterland, R.A. and Garza, C. (2002) Early postnatal nutrition determines adult pancreatic glucose-responsive insulin secretion and islet gene expression in rats. J. Nutr. 132 (3), 357–364.

Chu, K. and Tsai, M.J. (2005) Neuronatin, a downstream target of BETAA/NeuroD1 in the pancreas. Is involved in glucose-mediated insulin secretion. Diabetes 54 (4), 1064–1073.

Keverne, E.B. (1997) Genomic imprinting in the brain. Curr. Opin. Neurobiol. 7 (4), 463–468.

Hirasawa, R. and Feil, R. (2010) Genomic imprinting and human disease. Essays Biochem. 48 (1), 187–200.

Beydoun, H. and Saftlas, A.F. (2008) Physical and mental health outcomes of prenatal maternal stress in human and animal studies: a review of recent evidence. Paediatr. Perinat. Epidemiol. 22 (5), 438–466.

Jensen, C.H. et al. (2001) Neurons in the monoaminergic nuclei of the rat and human central nervous system express FA1/dlk. Neuroreport 12 (18), 3959–3963.

Crespi, B. and Badcock, C. (2008) Psychosis and autism as diametrical disorders of the social brain. Behav. Brain Sci. 31 (3), 241–261. discussion 261–320.

Middeldorp, C.M. et al. (2008) Life events, anxious depression and personality: a prospective and genetic study. Psychol. Med. 38 (11), 1557–1565.

Bauer, M. et al. (2008) Delta-like 1 participates in the specification of ventral midbrain progenitor derived dopaminergic neurons. J. Neurochem. 104 (4), 1101–1115.

Kozlov, S. et al. (2007) The imprinted gene Mgfl2 regulates normal circadian output. Nat. Genet. 39 (10), 1260–1272.

Murtagroyd, C. et al. (2009) Dynamic DNA methylation programs persistent adverse effects of early-life stress. Nat. Neurosci. 12 (12), 1559–1566.

McGowan, P.O., Meaney, M.J. and Szyf, M. (2008) Diet and the epigenetic (re)programming of phenotypic differences in behavior. Brain Res. 1237, 12–24.

Gluckman, P.D. and Hanson, M.A. (2007) Developmental plasticity and human disease: research directions. J. Intern. Med. 261 (5), 461–471.

Delahaye, F. et al. (2008) Maternal perinatal undernutrition drastically reduces postnatal leptin surge and affects the development of arcuate nucleus proopiomelanocortin neurons in neonatal male rat pups. Endocrinology 149 (2), 470–475.

Davies, W., Isles, A.R. and Wilkinson, L.S. (2005) Imprinted gene expression in the brain. Neurosci. Biobehav. Rev. 29 (3), 421–430.

Charalambous, M., Tsekoura da Rocha, S., Rowland, T., Ferrón, S.R., Ito, M., Radford, E., Schuster-Gossler, Hernández A. and Ferguson-Smith, A.C. Impaired thermogenesis and persistent hypothyroidism caused by the altered dosage of imprinted genes. submitted for publication.

Curley, J.P. et al. (2005) Increased body fat in mice with a targeted mutation of the paternally expressed imprinted gene Peg3. Faseb J. 19 (10), 1302–1304.

Chen, M. et al. (2009) Gsalpha deficiency in skeletal muscle leads to reduced muscle mass, fiber-type switching, and glucose intolerance without insulin resistance or deficiency. Am. J. Physiol. Cell Physiol. 296 (4), C930–C940.