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SYMPOSIUM: TROPHOBLAST DEVELOPMENT
REVIEW

The placental imprintome and imprinted gene function in the trophoblast glycogen cell lineage

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Louis Lefebvre obtained a BSc in biochemistry from the Université Laval, Québec (1987) before moving to the University of British Columbia for a PhD in biochemistry and molecular biology (1994) under the supervision of the Nobel Laureate Michael Smith, studying transcription regulation in yeast. He then completed two post-doctoral fellowships studying genomic imprinting in the mouse, first with Azim Surani at the Gurdon Institute, Cambridge (1998), then with Andras Nagy at the Samuel Lunenfeld Institute in Toronto (2003). He was recruited back at the University of British Columbia in Vancouver in 2003, where he is an associate professor in medical genetics and holds a Canada Research Chair.

Abstract Imprinted genes represent a unique class of autosomal genes expressed from only one of the parental alleles during development. The choice of the expressed allele is not random but rather is determined by the parental origin of the allele. Consequently, the mouse genome contains more than 100 genes expressed preferentially or exclusively from the maternally or the paternally inherited allele. Current research efforts are focused on understanding the molecular mechanism of this epigenetic phenomenon as well as the biological functions of the genes under its regulation. Both theoretical considerations and experimental results support a role for genomic imprinting in the regulation of embryonic growth and placental biology. In this review, recent efforts to establish the complete set of genes showing imprinted expression in the mouse placenta are first discussed. Then, the evidence suggesting that imprinted genes might be implicated in the emergence, maintenance and function of trophoblast glycogen cells is presented. Although the origin and functions of this trophoblast cell lineage are currently unknown, the analysis of mutations in imprinted genes in the mouse are providing new insights into these issues. The implications of this work for placental pathologies in human are also discussed.

KEYWORDS: genomic imprinting, imprintome, placenta, RNA-seq, trophoblast glycogen cells

Introduction

Genomic imprinting is an epigenetic phenomenon caused by the direct inheritance at fertilization of DNA methylation marks from a single parental gamete (Ferguson-Smith, 2011). Consequently, specific CpG-rich sequences in the genome acquire parent-of-origin specific DNA methylation marks, maintained as differentially methylated regions in somatic cells of the embryo. The main consequence of these parental allele-specific epigenetic modifications is to guide the expression of specific genes from a single allele, depending on its origin (Henckel and Arnaud, 2010). The

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mouse genome contains close to 100 of these so-called imprinted genes, with approximately the same number of maternally and paternally expressed genes, referred to here as MEG and PEG (Williamson et al., 2011). Early embryological experiments have shown that appropriate expression of imprinted genes is required for normal development. By nuclear transfer experiments, it was found that gynogenetic embryos, developed from fertilized diploid eggs containing two sets of maternal chromosomes, and androgenetic embryos, containing two paternal haploid complements, both fail to develop past mid-gestation (Barton et al., 1984; Mcgrath and Solter, 1984; Surani et al., 1984). This established that maternally and paternally inherited chromosomes in the zygote are not functionally equivalent and led to the suggestion that some genes are marked or imprinted differentially in the germlines, leading to their monoallelic expression, from a single parental allele. The phenotypic description of these reconstituted uniparental embryos also revealed some striking differences between androgenetic and gynogenetic embryos with regard to the development of their extra-embryonic tissues (Barton et al., 1984; Surani et al., 1984). Whereas gynogenetic embryos, as well as the similar parthenogenetic embryos derived from unfertilized activated eggs (Kaufman and Gardner, 1974; Surani and Barton, 1983), exhibited embryonic growth retardation but otherwise appeared relatively well developed, their extra-embryonic tissues, mostly the trophoblast lineage, is particularly stunted in these embryos. Conversely, androgenetic conceptuses exhibit severe defects in the embryo proper despite the presence of abundant trophoblast cells (Barton et al., 1984).

These experiments with uniparental embryos elegantly demonstrated that both maternal and paternal sets of chromosomes are required for normal development of the trophoblast lineage, but could not establish the number of genes regulated by this postulated imprinting mechanism. In fact, the androgenetic and gynogenetic phenotypes could have been attributable to a single imprinted gene, silent in one kind of uniparental embryos and expressed at higher dosage in the other. The demonstration that several autosomal loci are indeed regulated by imprinting came from genetic studies of mice carrying partial or complete uniparental disomies for specific chromosomes (Cattanach and Beechey, 1997; Cattanach and Kirk, 1985). These studies defined more than 10 regions of mouse autosomes for which biparental inheritance is required for normal development or post-natal survival. They also highlighted that the phenotypic consequences of uniparental disomies often implicate some aspect of embryonic and/or placental growth regulation (Williamson et al., 2011). These embryological and genetic studies have now been corroborated by the identification of more than 100 imprinted genes, some of which have been shown to play essential roles in the placenta (Coan et al., 2005; Fowden et al., 2011).

In addition to this experimental evidence for a role of at least some imprinted genes in placental development, a theoretical framework has been proposed to explain the emergence of imprinting during evolution. This parental conflict, or kinship theory, predicts that imprinting can evolve at developmental genes implicated in the regulation of energy exchanges between the pregnant mother and her offspring. More specifically, PEG are predicted to act as growth promoters, while MEG will act to restrict the mother's energy expenditure (Haig, 2000; Haig and Graham, 1991; Moore and Haig, 1991). Since the effects are likely to be manifested over relatively long periods of gestation and only in species in which maternal investment in development perdures post fertilization, imprinting is expected to be absent in oviparous species, at least with regards to maternal–fetal growth interactions. Consequently, there is a lot of interest in studying the evolution of imprinting in species situated at varying levels in the continuum between oviparity and viviparity. Currently, there is evidence for conservation of imprinting of some genes in marsupials, which do have rudimentary placentae, although not in egg-laying mammals (Renfree et al., 2008). Because of these considerations, as well as the implication of imprinted genes in human pathologies and placental function (Frost and Moore, 2010; Piedrahita, 2011), it is important to identify all the genes regulated by genomic imprinting during development, and notably in the placenta, and to invest more efforts in carefully characterizing the biological and developmental function of these genes.

This review first considers technical challenges to be considered when analysing imprinted gene expression in the placenta, an organ in which fetal-derived and maternal cell lineages are intimately amalgamated. Experimental approaches to circumvent these difficulties are discussed and a recent study re-investigating the imprinted status of MEG is presented. This leads to a discussion of new results aimed at defining the entire set of imprinted genes in the mouse placenta. From there, the focus shifts to the development of the trophoblast glycogen cell lineage. The characterization of mutations affecting a number of imprinted genes have revealed a role for these genes in the development of this lineage. Although the origin and function of trophoblast glycogen cells is currently unknown, these results implicate imprinted gene function in this lineage, raise some new questions about the interactions between different layers of the placenta and provide a basis for further characterization of trophoblast biology.

Maternally expressed imprinted genes in the placenta

A specific technical challenge with the identification of MEG in the placenta stems from the intimate relationship between cells of embryonic and maternal origin within this organ. In addition to the obvious possible contamination with maternal decidual cells, much of the murine placenta essentially bathes in maternal blood. As a result, false positives in the identification of placental-specific MEG can easily occur. This potential confounding problem and experimental approaches to circumvent it have been discussed recently (Proudhon and Bourc'his, 2010). These authors proposed two alternative strategies to standard reciprocal crosses to identify the presence of maternal contaminants: backcrosses (BB conceptus from AB $\times$ BB backcross) and embryo transfer (AB conceptus into CC female). Of these two strategies, only the latter allows the determination of imprinting status, the former simply providing a control to assess the presence of maternal contaminants. On the basis of the experimental evidence available at the time, they drew a list of 13 placen-