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Familial Hydatidiform Molar Pregnancy: The Germline Imprinting Defect Hypothesis?

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Abstract

Imprinting is the uniparental expression of a set of genes. Somatic cells carry two haploid sets of chromosomes, one maternal and one paternal, while germ cells contain only one of the two forms of chromosomes, male or female. This implies that during early embryogenesis the cells committed for developing the future germ cell lineage, the primordial germ cells, which are diploid, have to undergo a total chromosome reprogramming process. This process is delicately controlled during gametogenesis to ensure that males and females have only their respective form of gametes. The machinery involved in this process is yet poorly defined. Familial hydatidiform molar (HM) pregnancy is an abnormal form of pregnancy characterized by hydropic degeneration of placental villi and abnormal, or absence of, embryonic development. To date, the molecular defect causing this condition is unknown. However, in a few studied cases, the presence of paternal methylation patterns on the maternal chromosomes was observed. In this chapter, we summarize what is known about methylation aberrations in HMs and examine more closely the proposed hypothesis of a maternal germline imprinting defect.

1 Introduction: The Life Cycle of an Imprint

In the process of fertilization, both male and female gametes contribute equal amounts of genetic material to the newly formed zygote; however, the two haploid genomes (in the gametes) are not functionally equal (Walter and Reik 2001; Ferguson-Smith and Surani 2001). A set of genes is marked for silencing of transcription in one of the gametes but transcribed from the other. These sets of marked genes are said to be imprinted. Imprinting in somatic tissues is defined as mono-allelic transcription of a given gene depending on the parental origin of the chromosome. The imprinting process defines

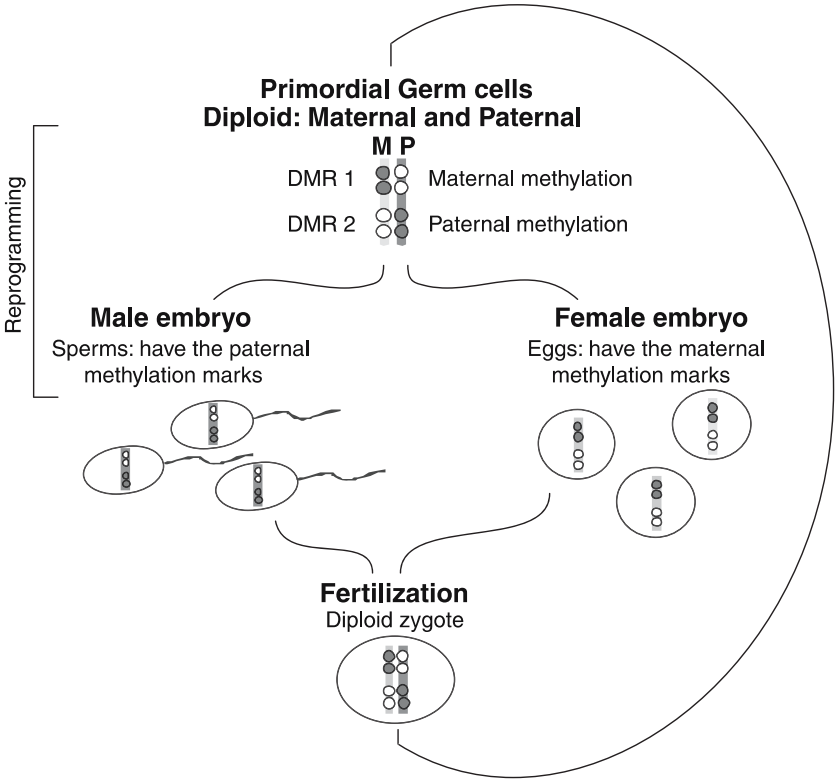

Fig. 1 A diagram showing the cycle of reprogramming of parental chromosomes during gametogenesis with respect to CpG methylation marks. Maternal alleles are shown in *light gray* while paternal alleles are in *dark gray*. *Open* and *filled circles* on the alleles represent unmethylated and methylated sites, respectively

the asymmetry between the two gametes and implies that the primordial germ cells, which are still diploid and carrying both maternal and paternal chromosomes in both sexes, have to undergo a reprogramming process to reflect the sex of the newly formed embryo (Hajkova et al. 2002, Li E 2002, Yamazaki et al. 2003; Fig. 1).

One unique example in humans for a disease that is manifested, or caused, by an imprinting defect is recurrent familial hydatidiform moles (HMs) (OMIM 231090). HMs mimic uni-parental mouse embryos (Barton et al. 1984) where androgenotes develop normal extra-embryonic tissues but there is no or little embryonic development, while parthenogenotes, on the other hand, give rise to the opposite phenomenon, normal embryonic development with poor development of extra-embryonic tissues. The exact molecular mechanism leading to familial HM is currently unknown. In this chapter, we will discuss the reasons that led investigators to suggest that it is a maternal germline defect in establishing the maternal imprinting marks and the validity of this hypothesis.

2
Familial Hydatidiform Molar Pregnancy

2.1
Diagnosis and Clinical Manifestations of Molar Pregnancies

HM is an abnormal form of human pregnancy characterized by hydropic degeneration of placental villi with the absence of, or abnormal, embryonic development. Based on the histology of the evacuated molar tissues, HMs are divided into two types: complete hydatidiform moles (CHMs) and partial hydatidiform moles (PHMs). CHMs are characterized by hydropic degeneration of all villi and absence of embryo, cord, and amniotic membranes. All the villi are (1) enlarged with cisternae, (2) avascular, and (3) surrounded by areas of trophoblastic proliferation. PHMs are characterized by focal trophoblastic proliferation with a mixture of normal-sized villi and edematous villi. The trophoblastic proliferation is less pronounced than in complete moles. An embryo, cord, and amniotic membranes are usually present in partial moles (Copeland 1993; Bonilla-Musoles 1993). This subdivision is supported by karyotype data, which show that most complete moles are diploids while partial moles are triploids. We note that moles are not always easily divisible into partial and complete moles; in a minority of cases, embryonic tissues are found in complete moles evacuated at early stages (Zaragoza et al. 1997; Fukunaga 2000) and some partial moles are found diploid with biparental origin.

2.2 Epidemiology and Genetics of Molar Pregnancies

The most recent reports estimate that 80% of CHMs have a diploid genome and are androgenetic. Among those, 60% are monospermic and 20% are dispermic (Kovacs et al. 1991; Lindor et al. 1992). The remaining 20% have a biparental genomic contribution to their genome. Most reported cases of HMs are sporadic and not recurrent. Occasionally, recurrent cases have been reported in one family member (Patek and Johnson 1978; Neumann 1980; Thavarasah and Kanagalingam 1988; Narayan et al. 1992; Tuncer et al. 1999; Ozalp et al. 2001; Fisher et al. 2000) and in a few cases, in at least two related women (familial cases) (Ambani et al. 1980; La Vecchia 1981, Parazzini et al. 1984, Seoud et al. 1995; Kircheisen and Schroeder-Kurth 1991; Sensi et al. 2000; Judson et al. 2002; Fisher et al. 2002; Al-Hussaini et al. 2003; Hodges et al. 2003; Fallahian et al. 2003; Agarwal et al. 2004; for review see Fisher et al. 2004). In several of these cases, women with recurrent moles had also abortions at various gestational stages and some achieved normal pregnancies and gave birth to healthy babies (Ambani et al. 1980; Seoud et al. 1995; Fallahian et al. 2003).

Consanguineous marriages were observed in many of these families, and in all of them the segregation of the defect is compatible with an autosomal recessive mode of transmission, with the women having recurrent moles being homozygous for the defective locus.

One group characterized the parental contribution to familial moles and demonstrated, using homozygosity mapping, that a maternal locus mapping to 19q13.4 between markers D19S924 and D19S890 is responsible for this condition (Moglabey et al. 1999). This locus was confirmed by other groups and on several families that allowed narrowing down the candidate region to 1.1 Mb flanked by markers D19S418 and AAAT11138 (Sensi et al. 2000; Hodges et al. 2003). However, not all families show linkage to 19q13.4, indicating the genetic heterogeneity of this disease (Judson et al. 2002; Slim et al. 2005), which could also reflect heterogeneity in the molecular mechanisms leading to familial moles.

2.3 Methylation Analysis in Molar Tissues

Methylation of DNA at the cytosines' fifth carbon is the most abundant modification of DNA in the human genome. This fifth base (5-methyl-cytosine: 5mC) occurs at a frequency of about 3%–4% of total cytosines. Most 5mCs occur at clusters called CpG islands. These are found in the promoter region of about one-third of human genes. These CpG islands play an important

role in the regulation of gene activity and expression of the nearby genes. Together with other epigenetic signals such as histone acetylation/methylation, they impose an open or closed chromatin structure that is associated with expressed (on) or repressed (off) gene expression. Regions that are actively transcribed (euchromatin) have promoter regions with mostly unmethylated CpG sites, acetylated histone tails and methylated lysine 4 on H3 histone subunits, while transcriptionally silent regions (heterochromatin) have mostly methylated CpG sites, deacetylated histones and methylated lysine 9 on H3 subunits (Fournier et al. 2002; Tamaru and Selker 2001). Imprinted genes that make the asymmetry in gene expression between the two sets of male and female gametes, and thus the two parental sets of chromosomes, are associated with differentially methylated regions (DMRs). These DMRs are CpG-rich regions that are heavily methylated on the non-expressed (repressed) allele and nearly devoid of methylation on the expressed allele.

The importance of correct methylation settings in the gametes and early embryogenesis is illustrated by the facts that aborted cloned animals (following nuclear transfer) show irregular methylation patterns (Kang et al. 2001; Beaujean 2004; Chen et al. 2004; Jaenisch 2004). Low methylation levels in sperm were also found to give lower rate of pregnancy in assisted reproductive techniques (Benchaib et al. 2005). Molar pregnancies—whether androgenetic or biparental (sporadic or familial)—are identical at the histopathological level; the only known functional difference between the maternal and the paternal genome is in the expression of imprinted genes. This has led to a common belief that imprinted genes play an important role in the pathology of moles and that a defective gene causing their deregulation could underlie the etiology of familial biparental molar pregnancies.

The above hypothesis was first tested by Judson et al. (2002) who studied a single biparental molar tissue from a family with recurrent HM. In this study, the authors made a detailed analysis of a well-characterized set of DMRs associated with *H19*, *KCNQ1OT1* (*LIT1*) *SNRPN*, *PEG1*, *PEG3*, and four with the *GNAS1* locus. They showed that seven of the nine analyzed maternally methylated DMRs (at *KCNQ1OT1*, *SNRPN*, *PEG1*, *PEG3*, and two of the four *GNAS*) were not methylated. For the paternally methylated DMRs, again, not all of them behaved similarly; the *H19* DMR had a normal methylation level while the *NESP55* DMR (at the *GNAS1* locus) was completely hypermethylated, indicating that the maternal allele behaved like a paternal allele. In the above study, no DNA polymorphisms were used to track the parental origin of the abnormally methylated alleles in the molar tissue. Indeed, this is needed to identify the parental alleles and see whether abnormal methylation is affecting both of them or only one. Abnormal methylation at both parental alleles would indicate epigenetic changes

during the proliferation of the trophoblast; while an abnormal methylation exclusively on the maternal alleles may indicate a primary defect that could be traced in origin to the maternal defect leading to familial moles.

We also analyzed the methylation of four DMRs, two paternally methylated, *H19* and *NESP55*, and two maternally methylated, *SNRPN* and *PEG3*, in two molar samples from one family (El-Maarri et al. 2003). Using a quantitative method (El-Maarri et al. 2002, 2004), we found similar trends of abnormal methylation like the ones reported by Judson et al. (Fig. 2). The studied paternal methylation (at *H19* and *NESP55*) in the two molar samples [biparental complete hydatidiform moles (BiCHM) 9 and 16] were lower than that of androgenetic complete hydatidiform moles (AnCHMs) and higher than that of normal chorionic villi and total blood DNAs, while the maternal methylation (*SNRPN* and *PEG3*) were decreased. This suggests that portions of the maternal chromosomes are assuming a paternal methylation patterns.

To investigate whether the two parental alleles are affected by the abnormal DNA methylation, we looked for single nucleotide polymorphisms (SNPs) and identified informative ones in a number of DMRs in one or two molar tissues

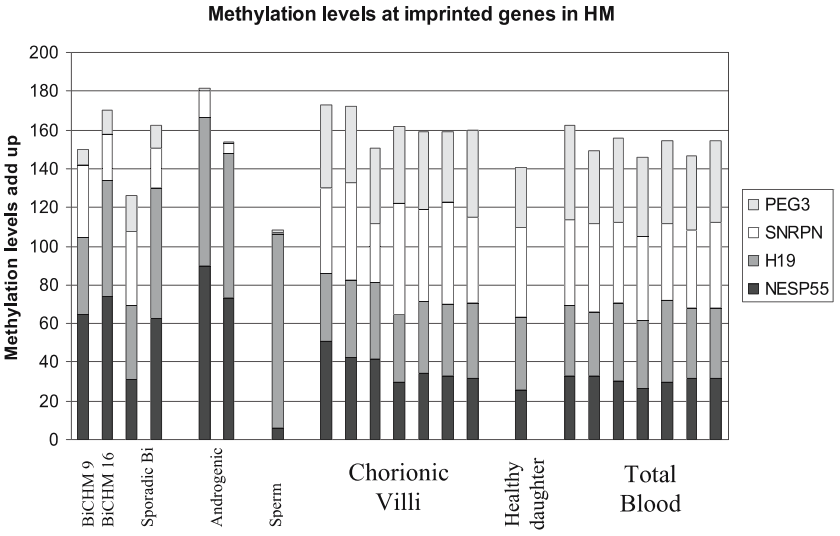

Fig. 2 The sum of methylation levels obtained at four imprinted genes in two molar tissues from two sisters (BiCHM9, BiCHM16) and a normal healthy daughter (Helwani et al. 1999). Analyzed samples include biparental sporadic and androgenetic cases, controls of normal sperms, chorionic villi, and total blood. The *lower two groups* represent paternal methylation; while the *upper two* represent maternal methylation. Data are reconstructed from El-Maarri et al. (2003)

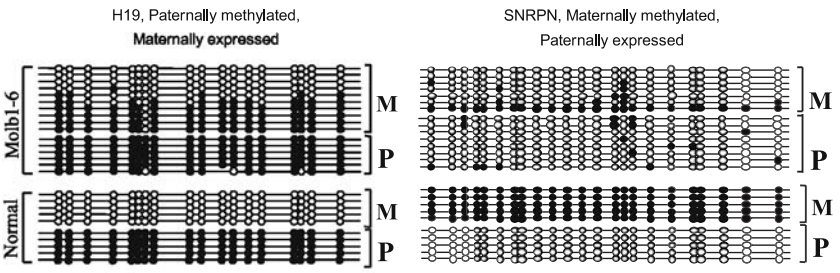

Fig. 3 A detailed methylation analysis by bisulfite sequencing from one molar tissue from family MoLb1 (sample *Molb1–6*). At both DMRs associated with imprinted genes, we have a considerable percentage of the maternal clones that acquired the paternal pattern of methylation (El-Maarri et al. 2003)

(*SNRPN* in BiCHM16; *NESP55* in *H19* in both BiCHM9 and BiCHM16). Bisulfite sequencing of individual clones at these DMRs (Fig. 3) showed paternal methylation pattern most maternal chromosomes; *H19* acquired methylation marks while *SNRPN* did not show methylation as it should on the maternal allele. This partial shift from the maternal to paternal patterns of methylation is intriguing and deserves to be investigated on additional imprinted genes. In case a similar shift is observed on all imprinted genes, this would indicate an abnormality in the setting or maintaining of the correct maternal methylation imprinting marks on the maternal chromosomes rather than a general failure in the methylation machinery. This is further supported by the fact that the two patients with recurrent HMs have both normal patterns and levels of methylation at the same four imprinted loci in their blood (El-Maarri et al. 2005).

2.4 Imprinted Gene Expression Analysis

Transcription analysis of imprinted genes in sporadic androgenetic moles showed abnormal imprinted gene expression and relaxation of imprinting in some androgenetic moles (Ohlsson et al. 1999; Ariel et al. 2000; Kim et al. 2003). These results are compatible with our data on androgenetic moles, in which we observed at *H19* a lower level of methylation than that observed in sperm DNAs. In familial biparental moles, only one study addressed the expression of one maternally expressed gene, $p57^{KIP2}$ (*CDNK1C*; Fisher et al. 2002). The authors used mouse monoclonal antibody against the $p57^{KIP2}$ protein on histological sections from familial and sporadic molar tissues. They demonstrated that $p57^{KIP2}$, which is expressed in normal first trimester placenta, is not expressed in biparental moles (familial and sporadic) nor in androgenetic moles.

2.5 Hypothesis of a Germline Imprinting Reprogramming Defect

Familial biparental HM pregnancy could be regarded as a disease of imprint reprogramming that takes place in the affected females to produce female gametes with paternal methylation imprints. However, to date there is no direct proof for such hypothesis mainly because of the impossibility of studying germ cells from such patients. Hereon, we list the reasons/observations that support such a hypothesis as well as reasons against it.

As indirect support for the germline imprinting defect hypothesis involving the maternal chromosomes we could list: (1) the fact that at both gross morphology and histology levels both familial biparental moles and androgenic moles are undistinguishable; (2) the similarity in the pattern of growth between biparental or androgenetic moles with that of experimentally created mouse androgenotes with two male pronuclei; (3) methylation analysis of the few available molar samples revealing that differentially methylated regions associated with imprinted genes show variable degree of paternal methylation patterns only on the maternal alleles; (4) the fact that only methylation at imprinted loci seem to be affected [the analysis of two X-linked genes (Judson et al. 2002; El-Maarri et al. 2003) revealed that they are normally methylated].

Reasons that could argue against a maternal germline imprinting defect are: first, all performed studies on molar tissues were done on samples of 6–14 weeks of gestation in which several changes could have occurred since fertilization, mainly because of the dynamic nature of early trophoblast and the postzygotic methylation changes that take place between fertilization and implantation; second, molar pregnancies are benign tumors of the trophoblast, and several studies have shown gain or loss of methylation marks at several imprinted genes including *PEG3*, *PEG1*, *SNRPN*, and *H19* in a variety of tumors. In colorectal cancer and Wilms' tumors, a similar shift from a maternal methylation pattern to a paternal one was observed at the *H19-IGF2* imprinted region (Steenman et al. 1994; Moulton et al. 1994; Taniguchi et al. 1995; Maegawa et al. 2001; Cui et al. 2001; Nakagawa et al. 2001); third, studies on sporadic, (androgenetic and biparental) moles demonstrated abnormal methylation or/and expression of a number of non-imprinted genes including oncogenes, tumor suppressors, and genes involved in protein synthesis, cell cycle, and intercellular communication (Olvera et al. 2001; Kato et al. 2002; for review see Li et al. 2002; Batorfi et al. 2003; Durand et al. 2003; Xue et al. 2004). It would be expected that at least some of these genes are also deregulated in familial biparental moles. The presence of normal methylation levels on two X-linked genes in familial biparental moles does not allow reach-

ing a conclusion on the methylation status of non-imprinted genes. A more comprehensive analysis of a large number of non-imprinted genes in molar tissues is needed.

2.6 Variability of Phenotype

One important observation derived from the methylation analysis on MoLb1 is that the abnormalities in the level of methylation was not the same at all loci and in all samples. This may also be true in other cases but could not be seen since only one molar tissue was analyzed (Judson et al. 2002). This also may be allelic and restricted to some families where variability in the phenotype of the conceptuses of these patients ranged from complete moles to spontaneous abortions at various developmental stages, and normal birth. This variability could be explained by the contribution of other environmental or/and genetic factors to the disease phenotype.

3 Concluding Remarks

Familial HM pregnancy is manifested by abnormal imprinting methylation marks. This abnormal pregnancy reflects the importance of establishing and maintaining the correct methylation marks for normal embryogenesis. The gene defect underlying this disorder is still to be identified; when identified it will increase our understanding of the protein machinery involved in the setting and maintenance of imprinting during embryogenesis and will answer the question as to when and how this abnormal paternal methylation was acquired in these tissues.

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