Genetics and Pathogenesis: A Recent Update

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3.1 Introduction

Gestational trophoblastic diseases (GTDs) include a spectrum of diseases, ranging from premalignant hydatidiform moles (complete and partial) to invasive neoplasms (called gestational trophoblastic neoplasms, GTN) including invasive mole, placental site trophoblastic tumor (PSTT), epithelioid trophoblastic tumor (ETT), and choriocarcinoma. PSTT is derived from implantation site trophoblastic tumor while ETT from chorionic-type intermediate trophoblast [1]. GTDs are unique as these lesions are derived not from patient tissue, but from the conceptus [1].

As a zygote matures into a blastocyst, its peripheral layers differentiate into cytotrophoblast and syncytiotrophoblast. The latter invades into the endometrium and uterine vasculature to form the placental tissue. Uncontrolled proliferation and invasion result in the group of disorders known as GTDs [2].

Partial hydatidiform moles (PHM) are biparental and are formed when a single ovum is fertilized by two (commonly) or rarely, one sperm. Their triploid genome is composed of two sets of paternal chromosomes and a single set of maternal chromosomes with a karyotype of 69, XXX, 69, XXY, or 69, XYY [3]. Occasional cases of tetraploid PHMs are also reported, which appear to result from trispermic fertilization of a single ovum [4].

In contrast, complete hydatidiform moles (CHM) are diploid and uniparental. Kajii and Ohama in 1977 first revealed the androgenetic origin of CHMs [5]. More commonly (in around 80% cases), they occur as a result of duplication of genetic material of a single sperm and in around 20% cases, dispermy is responsible. They have a 46, XX or 46, YY karyotype. Rarely, however, CHMs are biparental. Jacobs

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et al. in 1982 reported one such case. Biparental CHMs have been found in rare families whose females present with recurrent molar pregnancies [6]. These are known as familial recurrent biparental HMs (FBHMs).

3.2 Maternal DNA in CHMs

Though CHMs are known to be androgenetic, their mitochondrial DNA has been shown to be of maternal origin [7]. Hence theories hypothesizing the fertilization of anucleate eggs by one or two sperms were put forward. However, recent theories suggest post-zygotic diploidization of a triploid conceptus. As per these theories, a biparental zygote is first formed by fertilization of a single egg (M) by one (P1) or two sperms (P1 and P2) resulting in a triploid genome (MP1P1 or MP1P2). This triploid zygote then undergoes abnormal mitosis resulting in 1n, 2n, or 3n derivatives. Since paternal centrioles guide the first mitotic division of a zygote, the presence of two sets of active centrioles often results in abnormal mitoses. So a triploid zygote can result into MP1 (2n) + P2 (1n) or M (1n) + P1P2 (2n) or M + P1 + P2. The 1n outcomes can duplicate their genome to result in androgenetic diploid moles and the 2n P1P2 outcomes can directly produce diploid CHMs. All these would carry maternal mitochondrial DNA as cytoplasmic organelles would have been derived from the egg [3, 8].

The absence of maternal nuclear DNA (more specifically chromosome 11) in CHMs is the basis for p57KIP2 immunostaining. p57KIP2 is transcribed from a paternally imprinted maternally inherited gene CDKN1C [3]. It is expressed in the villous stromal cells of normal placenta. Hence its absence in the villous stromal cells of CHMs can help in differentiating them from PHMs and non-molar pregnancies (which show retained p57KIP2 expresssion) [3]. Occasional cases of PHMs with loss of p57KIP2 due to loss of maternal chromosome 11 have been reported [9]. Retention of maternal chromosome 11 in CHMs can result in positive p57KIP2 immunostaining, which may lead to misdiagnosis [10, 11]. Another paternally imprinted maternally expressed gene is IPL/TSSC3 (imprinted in placenta and liver) whose protein product IPL is absent from cells of CHM [3]. However, since it is not expressed in normal placental cells, its immunostaining, unlike p57KIP2, cannot be used for detection of CHMs.

3.3 Familial CHMs

Familial recurrent biparental hydatidiform moles (FBHM) occur due to mutations in a gene located on 19q13.4, which are inherited in an autosomal recessive manner [12]. These mutations affect the NLRP7 protein, a cytoplasmic protein that belongs to the CATERPILLAR group of proteins [13]. These proteins have an N-terminal pyrin domain, a NACHT domain, and a C-terminal leucine-rich repeat (LRR) domain. Most known mutations in NRLP7 cluster in the LRR domain, indicating that this region may have a crucial role in normal functioning of the protein [3] (Hoffner 2012). The mRNA of NLRP7 has been identified within the cytoplasm of

normal oocytes during oogenesis [13]. The precise function of NLRP7 is not clear, however, other proteins of the same family are known to have an important role in inflammation and innate immunity [14]. However, the exact relationship between NLRP7, inflammatory pathways, and HMs is as yet unclear. Abnormal methylation pattern of imprinted genes has been reported in CHMs [15]. Hence, recent research has been aimed at exploring the role of NLRP7 in the process of imprinting.

Imprinting is a reversible epigenetic process that results in silencing the expression of a set of alleles, which can be either maternal or paternal. Transcriptional silencing of one copy of certain genes occurs during gametogenesis. This is achieved by methylation of promotor region [16]. Such genes have only one functional copy in the offspring and any mutation arising within this copy can result in diseases.

Abnormal methylation patterns have been suggested to play a role in familial moles, which could be the result of either inherited abnormalities (involving inherited failure to pass on maternal imprint) or can arise as a result of de novo germline mutations [17, 18]. Owing to the paternal methylation patterns in maternally imprinted genes, FBHMs have a functional overexpression of the paternal genome [15]. The resultant phenotype is similar to androgenetic CHMs (with two copies of paternal chromosomes and no maternal chromosomes). Hayward et al. demonstrated a multilocus maternal imprinting defect in four families with FBHM who had biallelic NLRP7 mutations [19]. These data suggest that at least one of the functions of NLRP7 might be to establish the normal maternal imprinting patterns during embryonic development [13, 20, 21].

In 15 patients with recurrent biparental CHMs, Parry et al. reported the absence of NLRP7 mutations. Three of these women had biallelic mutations of C6orf221, a member of reproduction-related gene cluster on chromosome 6 [22]. They reported no phenotypic differences in CHMs with NLRP7 and C6orf221 mutations [22]. As research on familial CHMs progresses, we are likely to better understand the role of these and possibly other genes in the pathogenesis of BFHMs.

3.4 Genetics of HM

Using microarray analysis, Kato et al. in 2002 demonstrated the expression profile of HMs. They found that genes involved in Ras-MAP kinase, JAK-STAT, and Wnt signalling pathways were upregulated in HMs, suggesting that growth factor or cytokine-mediated signalling pathways may be the mechanisms underlying the trophoblastic proliferation [23]. The downregulated genes include insulin growth factor binding proteins, IL-1, TNF receptor, and CD44 among others [23].

3.5 Role of Maternal and Paternal Sets of Chromosomes

An imbalance in the ratio of maternal and paternal chromosomes occurs in HMs. An excess of paternal chromosomes in the absence of maternal genes results in CHMs that are phenotypically characterized by marked trophoblastic excess and absence of embryo proper. On the other hand, in the presence of maternal chromosomes, an

excess of paternal chromosomes produces PHMs which show moderate degree of trophoblastic proliferation and allow for the development of fetus. Interestingly, an excess of maternal chromosomes, as seen in ovarian teratomas, allows for development of embryonic tissues but fails to develop extraembryonic tissue. These findings could suggest that the formation of an embryo proper requires maternal set of chromosomes, while the development of extraembryonic tissues (including trophoblast) is dependent on paternal chromosomes [4]. So when both parents provide equal and appropriate share of their genome, a normal fetus begins to develop.

3.6 Invasive Mole

Both PHMs and CHMs can progress to an invasive mole. These have the potential for local and metastatic spread [24]. Most invasive moles are diploid and are known to be dispermic in origin [25]. A high percentage of tetraploid cells has been reported in invasive moles [26].

3.7 PSTT and ETT

Like most GTNs, genetic data on PSTT and ETT is sparse, however, most PSTTs are reported to be diploid with occasional demonstrating tetraploidy [3]. Few authors have described the karyotype of PSTTs and the abnormalities include absence of Y chromosome, loss of heterozygosity (LOH) 7p11.2, LOH 8p12-p21, and gain of 21q [3]. Xu et al. successfully analyzed three cases of ETT by CGH and found no chromosome gains or losses in any of them [27].

3.8 Choriocarcinoma

Gestational choriocarcinoma is an aggressive tumor that occurs in patients with history of conception, including both molar and non-molar. More than half the cases occur post CHM [28]. The monoallelic genome of a CHM is susceptible to functional inactivation by one-hit kinetics. An additional mechanism of dysregulation of gene expression is by imbalance in the imprinted genes which occurs due to uniparental transmission of genes. These together would result in reduced expression or inactivation of tumor suppressor genes, which predisposes CHMs to malignant transformation [4].

Gestational choriocarcinomas are histologically similar to non-gestational choriocarcinomas, however, they carry a better prognosis and are more chemosensitive than the latter [29]. One plausible explanation is that a part of the genetic material of gestational choriocarcinomas is of paternal origin, hence making them immunogenic and more chemosensitive. They are considered to be partial or complete allografts (containing biparental and uniparental genome, respectively). In contrast, non-gestational choriocarcinomas are host derivatives and hence tend to have poor

immunogenicity and response to chemotherapy [3]. Among the gestational choriocarcinomas, those arising from molar pregnancies tend to fare better as compared with those that arise post non-molar conceptions [30].

The genetic makeup of choriocarcinoma has mostly been studied in cell lines and occasionally in fresh tumor tissue. A number of chromosomal alterations including gains, losses, and rearrangements have been detected including deletions of 7p12-q12.2, amplification of 7q21-q31, and loss of 8p12-p21 [31, 32] NECC1 (not expressed on choriocarcinoma 1) gene located on chromosome 4q11-q12 is a tumor suppressor gene, whose expression is reduced in choriocarcinoma cells while it is consistently expressed in normal placental tissue [33].

3.9 Recent Advances

As our understanding of the pathogenetic mechanisms underlying GTDs has improved, the focus has shifted toward application of this knowledge to diagnosis, management, predicting the progression of HMs to GTNs, and understanding chemoresistance. Owing to the rarity of GTNs and paucity of tissue samples (being highly vascular tumors, biopsy is relatively contraindicated in GTNs and most cases directly undergo therapy), most of our understanding is based on research, which has been performed on preserved cell lines [34].

3.10 Genetics in Diagnostics

One of the characteristics used in FIGO risk scoring for GTDs is the interval from index pregnancy in months. Females who develop GTN after a longer interval from the index pregnancy tend to fare worse. However, to conclusively ascertain the index pregnancy becomes difficult in patients with multiple previous pregnancies. A comparison of microsatellite polymorphisms of the tumor with previous pregnancies can help in such cases [34]. Such comparison is also helpful to differentiate gestational from non-gestational choriocarcinomas and a molar versus non-molar origin of a choriocarcinoma, with both the distinctions carrying significant prognostic relevance [3]. Cases that present with a diagnostic uncertainty and where a non-gestational neoplasm is being considered as a differential diagnosis can also be worked up using such genetic analysis tools. This distinction is relevant as GTNs require prompt management with aggressive chemotherapy.

3.11 Predicting Progression to GTN

Approximately 10% CHMs transform to GTNs [35]. The risk of progression is higher for a dispermic CHM in comparison with a monospermic CHM, as suggested by a multitude of studies [25, 36, 37]. However, other studies have contradictory results [38–41]. No significant difference in risk of progression has been

reported between BFHMs and androgenetic CHMs [42]. Progression of PHM to GTN is controversial. Case reports of PHMs progressing to GTN do exist in the literature, however, a review of literature shows that the cases which progressed were diploid PHMs and hence could possibly represent misdiagnosed CHMs [41, 43, 44]. It is hence possible that the risk of developing a GTN post PHM is similar to that seen after a non-molar pregnancy [3].

Attempts to determine the factors involved in malignant transformation have been made by comparing the genetic signatures of normal placenta, HMs, and GTNs. However, owing to the rarity of the disease and the paucity of biopsy samples (since a biopsy for confirmation is not done and most cases are treated with chemotherapy without surgery), most research is based on in vitro studies. Activation of oncogenes and inactivation of tumor suppressor genes has been implicated in disease transformation [34]. Upregulation of genes like SET, NANOG, and STAT-3 as well as downregulation of genes including TIMP2, TIMP3, Kiss-1, E-cadherin, DCC-1, APC, beta-catenin, NECC1, caspase 8, caspase 10, and MASPIN have been found to be associated with higher risk of developing GTN from HMs [34].

The role of miRNA has also been explored in progression of HM to GTN. Choriocarcinoma tissues had significant under expression of miR-199b as compared with HMs [34]. Importantly, miRNA expression can be modified by proteins called siRNAs. Forced expression of miRNA-199b has been found to result in a reduction in cellular proliferation in a choriocarcinoma cell line [34].

Another epigenetic event relevant to invasive transformation of HMs is silencing of tumor suppressor genes by methylation of CpG regions. These regions are rich in cytosine and guanine residues and are clustered in the promotor region of various genes. Methylation of CpG islands results in transcriptional silencing of downstream genes. Smith et al. demonstrated that hypermethylation induced silencing of E-cadherin and p16 in HMs can result in invasive transformation [45].

These insights into the pathogenesis of transformation of HMs to GTNs would be clinically relevant when they could be used as biomarkers for identifying those HMs that are at a higher risk of invasive transformation. The current biomarker in use is serum beta hCG (human chorionic gonadotropin). It is a sensitive and relatively noninvasive test that is used for regular follow-up of women with history of HMs. It helps in early identification of cases that are transformed to GTNs. However, at the outset of diagnosis of HM, no current marker exists to identify those women who are likely to develop GTNs. Large prospective clinical trials need to be done on the above genetic markers in the hope of having such a biomarker in the future.

3.12 Genetics of Drug Resistance

GTNs are chemosensitive diseases with nearly 100% survival in FIGO low-risk cases and approximately 87% in high-risk cases [46, 47]. There is a paucity of studies on the exact pathogenetic mechanisms that underlie drug resistance in GTNs. Chen et al. studied cases of 5-fluorouracil and etoposide resistance and found reduced levels of proapoptotic protein PUMA in these cells. On introducing PUMA

using an adenovirus vector, an improvement in chemosensitivity was reported [48]. A knowledge of such mutations could theoretically be helpful in identifying at the time of diagnosis, those women whose disease is likely to be chemoresistant.

Recent research on newer drug targets in chemoresistant GTNs has brought into light MAPK (mitogen activated protein kinase) pathway. MAPK is a part of the signalling pathway involved in differentiation and migration. PSTTs demonstrate the active phosphorylated form of MAPK, in contrast to the inactive form found in normal placenta [49]. Treating a PSTT cell line (IST-2) with MAPK inhibitor (CI-1040 and PD 59089) resulted in significant reduction in motility and invasiveness of IST-2 cells. In contrast, the two inhibitors did not have any effect on normal extravillous trophoblastic cells [49].

Other targetable molecules include mTOR (mammalian target of rapamycin), other PI3K family members, and EGFR (epidermal growth factor receptor). These molecules have been found to be upregulated in GTNs including choriocarcinoma and hence they may serve as a therapeutic target [50, 51]. EGFR expression is significantly higher in choriocarcinoma placentae as compared to normal ones with a similar period of gestation [52]. A reduction in EGFR binding sites on choriocarcinoma cells was reported upon exposure to EGFR inhibitors [52]. However, most of this research is based on in vivo cell cultures and clinical trials would serve to provide more definitive results regarding their usefulness as therapy options in chemoresistant GTNs.

3.13 Future Perspective

Many aspects of GTNs remain which need to be explored by future research. To determine and validate biomarkers that can predict the progression of HMs to GTNs, prospective clinical trials need to be carried out. Such studies demand collaborations between the laboratory and clinics. To determine the factors underlying drug resistance and alternative regimens for chemoresistant cases, large clinical trials can be designed. Over longer term, it would be interesting if, at the time of diagnosis, those women can be identified who are at higher risk of progression and resistance to therapy.

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