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Parental imprinting of the human H19 gene

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It has only recently become clear that genetic imprinting plays an important role in human embryogenesis and in processes leading to the development of pediatric cancers and other human diseases. Using a unique human tissue, the androgenetic complete hydatidiform mole, we established that the maternally inherited allele of the imprinted H19 gene is expressed. Our results also show that the paternal allele of the human IGF-II gene, a gene suspected to be parentally imprinted in humans, is expressed.

H19; IGF-II; Human genomic imprinting; Hydatidiform mole; Androgenetic tissue

1. INTRODUCTION

It has only recently become clear that mammalian development is probably unique in its requirement for the functional presence of both maternal and paternal genomes [1]. Paternal and maternal genomes contribute in different ways to the developing feto-placental unit. When all the genetic material is maternally derived, early embryonic development is almost normal, while placental tissue is barely present. In contrast, embryos with paternally derived genomes show the reverse effect [2]. These observations can be explained on the basis of gene imprinting. The essence of the gene imprinting phenomenon is that only one allele out of the two alleles is expressed, depending upon its parental origin.

At least two genes on mouse chromosome 7, which are tightly linked, are parentally imprinted. These are the insulin like growth factor-II (IGF-II) which is expressed from the paternally derived allele [3], and the H19 gene, which is expressed from the maternally derived allele [4]. The function of the H19 gene is still unknown, and it has been suggested that it exerts its effect as an RNA [5]. The H19 and IGF-II genes map to the human chromosome 11p15.5 [6]. The H19 transcript is one of the most abundant mRNAs in the developing mouse embryo [5], in human embryonic tissues (Goshen et al. submitted) and in human placenta [8]. The IGF-II gene is highly expressed both in embryonic and extra-embryonic human tissues [9].

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Complete and partial hydatidiform moles are non-metastatic gestational trophoblastic diseases. The complete hydatidiform mole results from the fertilization of an 'empty egg', so that two normal haploid sets of paternal chromosomes are exclusively present [10] with no formation of an embryo [11]. A partial hydatidiform mole consists of both embryonal and hydatidiformic changes of the placental tissue [11]. The partial hydatidiform mole is endowed with two sets of paternal chromosomes and one set of maternally derived chromosomes [11]. Normal placenta, complete and partial hydatidiform moles consist of trophoblastic cells and express the genes coding for placental-specific proteins, such as the α and β subunits of the chorionic gonadotropin (CG) and the human placental lactogen (hPL) in vivo and in vitro [11,12].

Zhang and Tycko have recently shown [13] the monoallelic expression of the human H19 gene, but the system they used did not enable them to identify the parental origin of the expressed allele. The complete hydatidiform mole, with an exclusively paternal-derived genome, is a suitable biological system for determining the parental origin of the expressed human H19 allele. Here we show that the paternal genome of the molar tissue does not express the H19 gene, or expresses it at a very low levels, but it does express the IGF-II gene. Our findings, together with those of Zhang and Tycko [13], provide strong evidence in favor of parental imprinting of the human H19 gene, with the maternally derived allele as the active one. We have also shown that the paternally derived allele of the human IGF-II gene is expressed. This finding is of considerable interest because this gene is suspected to be parentally imprinted in humans.

2. MATERIALS AND METHODS

2.1. *Tissue sampling*

Placental specimens were collected from conception products derived from the termination of normal first trimester pregnancies, approved by the local ethical committee. Term placentae were derived from normal vaginal deliveries. Partial and complete hydatidiform moles were obtained by vacuum aspiration of the uterus. All specimens were frozen in liquid nitrogen and kept at −80°C for further analysis. Partial and complete hydatidiform moles were diagnosed by ultrasonographical studies, and by histopathological and cytogenetical analysis. A JAr cell line was maintained as previously described [14].

2.2. *Isolation of RNA*

Total cellular RNA was isolated from the various tissues by the guanidinium thiocyanate method [15] and from the cells by the guanidinium-thiocyanate/cesium-chloride method [16].

2.3. *Northern blotting*

10 μg of each RNA sample were separated by 1% agarose-formaldehyde gel electrophoresis and transferred to Hybond-N Nylon filters (Amersham, UK). The blots were stained with Methylene blue [17] and hybridized with 18 S ribosomal RNA (not shown), in order to ascertain that equal amounts of RNA have been loaded in each lane. The blots were prehybridized at 42°C in 50% formamide, 5× SSPE, 5× Denhardt's solution, 0.1% SDS and 0.1 mg/ml herring testes DNA and hybridized with specific cDNA probes. The probes used for hybridization were prepared from the appropriate clones of H19, IGF-II, CGα, CGβ and hPL. The probes were labeled according to the Random Primed Labeling Kit (Boehringer, Mannheim) protocol. The blots were washed twice in 0.1× SSC, 0.1% SDS at 65°C and exposed to AGFA Curix film at −80°C.

3. RESULTS AND DISCUSSION

Since normal placenta, partial and complete hydatidiform mole have a different genomic make up despite the common cell of origin (i.e. cytotrophoblast), we compared the expression of the H19 and IGF-II genes in these tissues. We have used a choriocarcinoma cell line (JAr) as a model for dividing cytotrophoblastic cells.

First trimester, third trimester placentae and partial molar tissue express H19 to a similar extent. The highest expression was fond in the choriocarcinoma cell line. In complete molar tissue, the H19 expression was maximally 10% of that in normal placental tissue and partial hydatidiform mole (Fig. 1A). The intensity of the H19 signal in the complete molar tissue was found to depend upon the extent of maternal tissue contamination. This contamination was due to the presence of maternal decidua, which was found to express the H19 gene to an extent very similar to that in normal placenta (not shown). However, we can not exclude the possibility that the faint hybridization signal we found with the RNA of the complete molar tissue is at least partly due to a very low expression of the gene in this tissue. Both explanations comply with the findings of Zhang and Tycko [13] who reported variable expression in the human placenta of the silent human H19 allele (the allele not expressed in embryonic tissues). Our finding that H19 is not expressed or is expressed at a very low level in an androgenetic tissue, in contrast to its high expression in the normal placenta, endowed with both one paternal and one maternal set of chromosomes, together with the report on its mono-allelic expression in humans [13], provides strong evidence for the parental imprinting of this gene in humans, with the active allele derived from the mother.

The IGF-II gene was expressed in all the tissues examined (Fig. 1B). Several mRNAs of the IGF-II gene are formed as a result of differential splicing [18]. In JAr cells only one of the IGF-II transcripts is expressed at the same level as in the other tissues.

Gene imprinting is considered to be a property of certain chromosomal areas [7]. The human IGF-II gene is tightly linked to the imprinted H19 gene on chromosome 11p15.5, an area synthenic with the distal part of mouse chromosome 7 which carries the imprinted mouse IGF-II [3] and H19 genes [4]. Gene imprinting fulfills important functions in the development of embryonic and extra-embryonic tissues which are conserved in mammals [1]. These observations support the notion that the human IGF-II gene is a likely candidate for a parentally imprinted gene. Our finding that IGF-II is expressed in the complete molar tissue, a tissue equipped with paternal chromosomes only, at least as strongly as in the normal placenta, means that, in case the parental imprinting of human IGF-II can be verified, the paternally derived allele of this gene is the only active one. The fact that the IGF-II gene, which is located within 100 Kb of the H19 gene, is expressed in complete hydatidiform mole makes the possibility that the H19 was not expressed because of loss of its locus in this tissue very remote.

In order to rule out the possibility that the absence of H19 expression is simply a matter of variability in tissue-specific expression, we have also determined the expression of the genes for the CGα and -β subunits and for hPL, all of which are well-established placental differentiation markers [12,19]. The gene for CGα is highly expressed in first trimester placenta, and at a much lower level in partial mole and in term placenta, but is not detectable in JAr (Fig. 1D, lanes 1,2,3,5, respectively). Complete hydatidiform mole expresses this gene at the same level as first trimester placenta (Fig. 1D, lane 4). Similar results were obtained when CGβ expression was measured, except that complete hydatidiform mole expresses this gene at a higher level than first trimester placenta (Fig. 1E). These findings are consistent with previous reports dealing with placental-specific gene expression [11,12,19]. The expression of CGα and CGβ genes was not detectable in JAr cells, however, during the differentiation of JAr cells in culture, increasing levels of mRNAs of these genes were measured [14]. The expression of hPL increases in the following order: partial molar tissue, complete mole, first trimester and term placenta (Fig. 1C, lanes 1,2,3,4, respectively). No

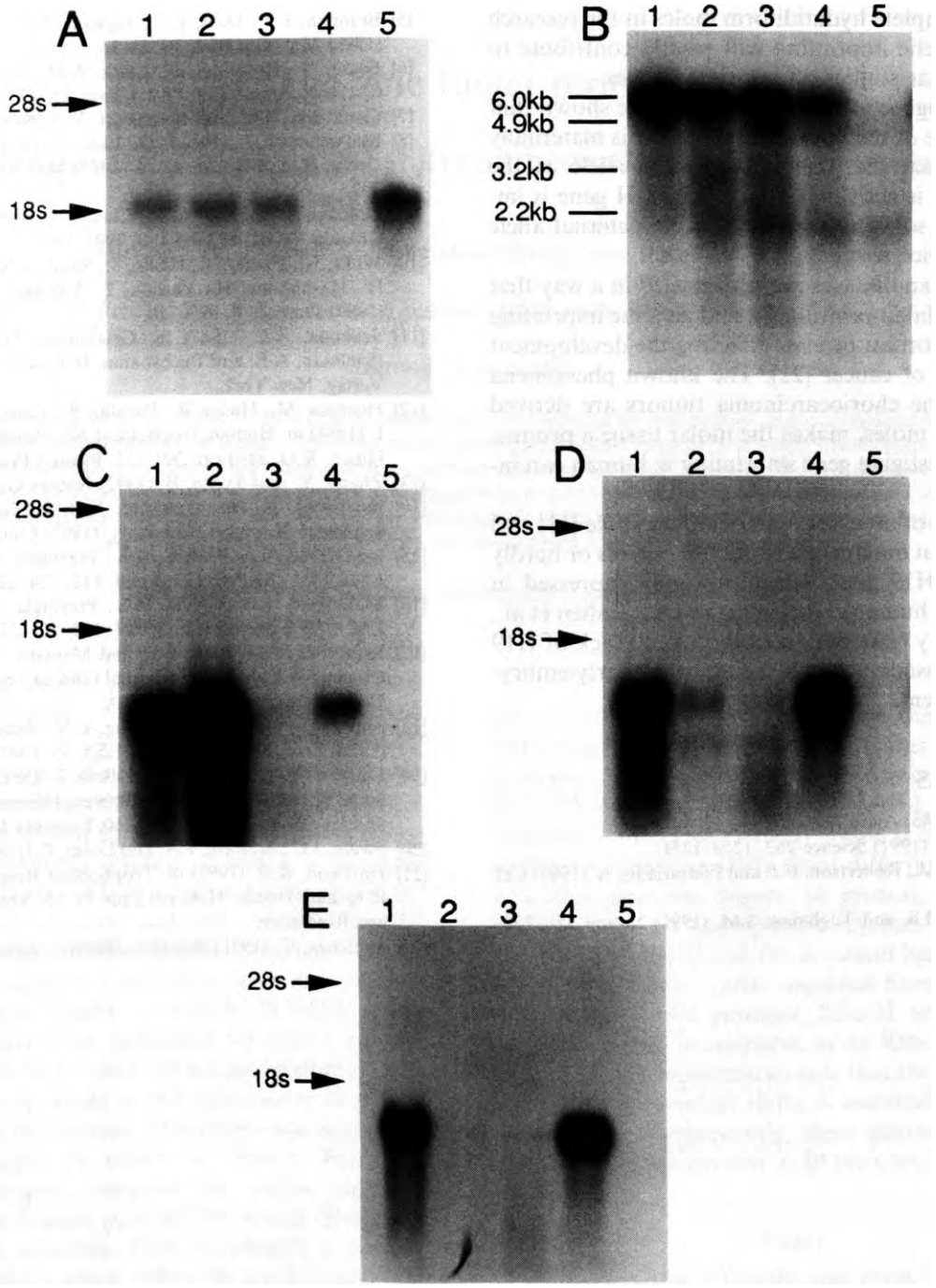

Fig. 1. Expression of H19 (A), IGF-II (B), hPL (C), CGα (D) and CGβ (E) in placental, hydatidiform mole tissues and in choriocarcinoma cells (JAr). Northern blots containing RNA isolates from first trimester placentae (n = 4; 12 weeks; lane 1), term placental tissues (n = 5; lane 2), partial hydatidiform mole (n = 3; 18 weeks; lane 3), complete hydatidiform mole (n = 3; 12 weeks; lane 4) and JAr cells (lane 5).

expression was detected in the JAr cells (Fig. 1C, lane 5, [14]).

In almost all the reports on parental imprinting in mice and humans the unique human androgenetic complete hydatidiform mole is mentioned, because the lack of embryonic tissue in this trophoblastic disease indicates the importance of parental gene imprinting in human embryogenesis [1,20]. To the best of our knowledge this tissue was never used to show whether monoallelic-expressed genes are maternally or paternally imprinted in men. In the case of genes expressed only during human embryogenesis and which are expressed in placenta, the use of the complete hydatidiform mole as a source of a paternally derived tissue offers an advantage in the identification of genes uniquely expressed from the paternally inherited chromosome. Our intro-

duction of complete hydatidiform moles in the research of human genetic imprinting will greatly contribute to future molecular studies on imprinted genes.

Using androgenetic molar tissue we have shown that the active allele of the imprinted H19 gene is maternally derived and that the paternally derived allele of the human IGF-II is active. The mouse IGF-II gene is imprinted and is solely expressed from its paternal allele in nearly all mice tissue [3].

Several human diseases are transmitted in a way that suggests parental imprinting [1], and genome imprinting may be an important process affecting the development of some types of cancer [22]. The known phenomena that 50% of the choriocarcinoma tumors are derived from complete moles, makes the molar tissue a promising tool to investigate gene imprinting in human carcinogenesis.

The absence of an embryo in complete mole [11] and our findings that molar tissue does not express or hardly expresses the H19 gene, which is highly expressed in several normal human embryonic tissues (Goshen et al., submitted), may raise the possibility that a lack of H19 expression is associated with the failure of early embryonic development.

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