Acta Genet Med Gemellol 45: 77-82 (1996)

Wiedman-Beckwith Syndrome, Tumorigenesis and Imprinting

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THE WIEDEMANN-BECKWITH SYNDROME (WBS)

WBS is an overgrowth malformation syndrome characterized by highly variable expressivity, associated with predisposition to different types of pediatric tumors including Wilms' tumor (WT), adrenocortical carcinoma (ADCC), rhabdomyosarcoma (RMS) and hepatoblastoma (HEP). Most cases are sporadic, however 15% of the cases are familial. Cytogenetic, genetic and molecular analysis of the different forms of this syndrome and associated tumors have provided increasing evidence that the gene (or genes) map to 11p15.5 and that genomic imprinting can account for the strange genetics of this syndrome/ tumors [1]. Two candidate genes, H19 and Igf2, which are both imprinted, but in opposite direction, map close to but not within one of the two smallest region(s) defined both by constitutional and tumoral rearrangements. These two genes, H19 and Igf2, and their specific parental imprint, may thus account for the pattern of inheritance observed, the variable expressivity, the specific loss of alleles and the loss of imprint. However, that these genes map 400 kb away from one cluster of breakpoints observed in the cytogenetic cases of WBS suggests that other genes could be involved. Indeed, although mapping to a different subregion, a sequence wit the properties of a tumor Suppressor (rhabdomyosarcoma cell line) has recently been isolated [2].

Furthermore, neither reduplication of the active Igf2 paternal allele nor relaxation of Igf2 imprinting is sufficient for tumorigenesis, thus indicating that other mutation(s) must occur. The phenotypic consequences of these aberrant expressions will be better understood when the tissues, the stage of development or the state of differentiation are precisely identified [3-5].

MONOALLELIC EXPRESSION

Expression of only one allele can be demonstrated by RT-PCR at the RNA level whenever a DNA polymorphism located in the expressed portion of the gene is available. By

comparison of the amplification at the DNA level, it is thus possible to demonstrate whether it is expressed from the maternal paternal allele. Monoallelic expression is usually limited to embryonal and fetal tissues.

Although it is not yet known whether each allele of a gene carries its own parental-specific imprint, by convention the silent allele is the imprinted one. When a gene is imprinted, only one of the two alleles is expressed, usually the same in several tissues. However, a switch of the imprint from one allele to the other has already been reported in a few instances. The term gametic imprinting should apply only to genes for which monoallelic expression has been demonstrated. Several types of observations are actually needed to confirm imprinting.

Allele specific methylation

Methylation patterns of the maternal and paternal alleles of these genes have been analyzed at different stages of development and in the gametes. Clear parent-specific patterns are observed both at the molecular level and probably at the chromosomal level. In mouse, four imprinted genes, *Mash2*, *Ins2*, *Igf2* and *H19* are closely linked on chromosome 7. In humans, the same organization is conserved in the region 11p15.5. Igf2 and H19 are imprinted in opposite directions, H19 being expressed from the maternal allele and Igf2 from the paternal allele. The paternal allele of H19 is hypermethylated for most CpG sites in its promoter region with only some of them being methylated in sperm. The mutually exclusive expression of these two genes seems to be the rule but there are exceptions which require further investigations to be fully understood. This pattern of expression is most probably functionally and mechanistically coordinated by intramolecular competition for a common locus element [6].

Chromatin compaction

The methylation differences have been correlated to different chromatin configurations revealed from an analysis of sensitivity to restriction endonucleases. Thus, in liver nuclei from mice embryos, only the chromatin of the expressed maternal H19 allele was found to be open in the promoter region. Surprisingly, however, the same open configuration was also observed in brain, a nonexpressing tissue [7].

Asynchronous replication

For most genomic DNA the maternal and paternal chromosome homologs replicate synchronously during S phase; however, all of the known imprinted sequences are imbedded within 1- to 2-Mb chromosomal regions that show differential replication, with the paternal allele usually replicating earlier than the maternal copy during each cell cycle [8] or in even more complex alternate patterns. Asynchronous replication has been demonstrated for the region carrying the H19 and Igf2 genes, but not for HBB which lies outside the WBS region(s), in both human and mouse.

Polymorphism and mosaicism

However, genomic imprinting can be polymorphic among individuals and even, though rarely, show allele switching. These variations may have important consequences on the clinical variability of genetic disorders and on the proneness to develop an embryonal tumor. This was recently demonstrated for WT1 and Igf2R [9,10].

Promoter specific imprinting

Genes may have different promoters which can be used alternatively, depending on the stages of development, differentiation or the tissue. In the case of Igf2, four different promoters may lead to the expression of mRNA of different lengths. It was recently shown that the P1 promoter is biallelically expressed while the other promoters are monoallelically expressed, always from the paternal chromosome. Thus, an aberrant switch of promoter can result in aberrant expression of either one or two alleles and therefore mimic relaxation of imprint [11].

Alternatively, relaxation of the maternal imprint of promoters P2-P4 could lead to biallelic expression.

CONSTITUTIONAL DEFECTS

As revealed by several biases in parent of origin and somatic mosaicism, constitutional defects in WBS can be due to germline, or early or late postzygotic alterations.

Chromosomal anomalies

Duplications of 11p15 are always of paternal origin while apparently balanced translocations involving 11p15.5 breakpoints are of maternal origin.

Familial transmission

In families, there is a large excess (3-fold) of carrier mothers due to reduced fertility of male carriers and reduced penetrance when transmission occurs from an affected male [12].

However, the role of Igf2 has not yet been established, since a recombinant has been detected in a WBS family [13].

Uniparental paternal disomy (UPD)

UPD can be defined as the presence of two chromosomes (or parts thereof) of the same pair from the same parent, i.e. the offspring lacks a chromosome from the other parent. UPD limited to discrete chromosomal regions has been recognized in human genetic disorders such as Prader-Willi syndrome (PWS), Angelman syndrome (AS) and WBS. The two chromosomes can either be identical (isodisomy) or different (heterodisomy). Heterodisomy necessarily proceeds from nondisjunction at meiosis I, whereas isodis-

omy can result either from non disjunction at meiosis II or from a postzygotic recombinational event. In WBS, uniparental isodisomies of 11p15.5 are of paternal origin and affect 20% of sporadic WBS cases. Most of these cases display somatic mosaicism with an increased risk of 64% (versus 10%) of developing a tumor associated with a paternal disomy of the same region. Most cases with UPD are associated with partial isodisomy for part of 11p and somatic mosaicism indicating postzygotic mitotic recombination. Depending on the stage at which this event occurred, and in which cell lineage, the proportion of cells and the type of tissue affected can vary widely and confer a different predisposition to develop a tumor [1, 14]. Indeed, the loss of maternal alleles in a proportion of these tumors represents the somatic equivalent of UPD. In a few cases of WT, partial UPD has been demonstrated in the normal kidney tissue surrounding the tumor [15, 16].

Constitutional relaxation of the imprint

A defect in genomic imprinting can occur constitutionally, leading to growth abnormalities and predisposition to WT. Relaxation of Igf2 imprinting in four of six fibroblast cultures from WBS patients has been detected [17]. In one child with generalized overgrowth, Igf2 was transcribed from both alleles in her kidney peripheral blood leukocytes and WT. This is consistent with constitutional relaxation of Igf2 imprinting arising from a germline mutation or as a very early event during embryogenesis. In contrast, kidney samples from nine children with normal growth profiles showed monoallelic transcription of Igf2 [18].

However, in a larger series of 42 WBS cases, Reik et al. [19] failed to detect alterations of the imprint in the lymphocytes of these patients, suggesting that sporadic WBS is not associated with a general alteration of methylation imprinting of the Igf2 and H19 gene. Thus constitutional alterations of the imprint may not be a frequent event to account for WBS (less than 10%).

TUMOR SPECIFIC ALTERATIONS

Similar alterations are seen in tumors, suggesting a kind of continuous occurrence of alterations from germ cells through development and into early childhood.

Maternal losses of alleles

The involvement of one or several imprinted genes mapping to the region 11p15.5 in pediatric embryonal tumors (WT, ADCC, RMS, HEP) has been suspected for quite a while. The losses of alleles or loss of heterozygosity (LOH), preferentially if not exclusively implicated the maternal chromosome. The regions for LOH contain two imprinted genes, Igf2, a growth factor expressed from the paternal chromosome, and H19, a tumor suppressor gene expressed from the maternal chromosome. LOH is detected in about 50% of the tumors.

Somatic relaxation of imprinted genes

In the remaining tumors without LOH, a relaxation of the imprint (LOI) of the maternal allele of Igf2 was observed in 2/3. In agreement with the coordinate expression of these two genes, the loss of the maternal imprint of Igf2 is associated with the silencing of H19. Thus, in the majority of tumors Igf2 is expressed (either from the paternal allele in tumors with maternal LOH or from both chromosomes in tumors with ROI), while H19 expression is abolished (either by loss of the active maternal allele or by hypermethylation of its promoter) [20]. This would confirm the tumor suppressor role of H19 [21].

DNA methylation: alterations and imbalance

There have been several reports showing alterations of methylation profiles in many types of tumor. These alterations can be either hypomethylation or hypermethylation depending on the region or gene examined. More likely, in cancer, local imbalance of the methylation profile can result in aberrant expression or silencing of a gene.

CONCLUSION AND PERSPECTIVES

Together, the two genes, Igf2 and H19, with their specific role and imprint can account for the unusual transmission profiles of familial WBS, the variable expressivity, the maternal LOH and the loss of the Igf2 imprint or extinction of H19. However, several observations suggest that other genes may be involved:

1. Igf2 and H19 map 400 kb from a cluster of breakpoints observed in WBS patients with maternal translocations;
2. recombinational events between Igf2 and WBS in familial cases, and
3. a new sequence with tumor suppressor activity has been isolated. Moreover, the fact that more than 10% of tumors do not show either of these imprinting and/or expression alterations suggests the involvement of other genes or more subtle mutations in regulatory sequences. In addition, neither the reduplication of the active paternal allele of Igf2 nor the relaxation of the inactive maternal allele are sufficient for tumor development.

Since imprinting is expected to start in the germline, a heritable molecular tag is needed. This tag should be able to regulate gene expression and be removable during each cycle of germ cell development. DNA methylation may be one of the molecular markers. Igf2 and H19 are closely linked in mice and humans, but are imprinted reciprocally. It has been proposed that expression of the two reciprocally imprinted genes, H19 and Igf2, is functionally and/or mechanistically related and that the imprinting of a single chromosomal site might control the activity of both genes [22].

It has been suggested that the observed methylation and condensed chromatin of the inactive paternal H19 promoter may be the controlling event. Imprinting of H19 and Igf2 might be linked mechanistically if the Igf2 and H19 promoters compete for the two enhancers downstream of H19 [7, 22]. The enhancers may activate Igf2 expression, but only if the H19 gene is methylated and inactive, as on the paternal chromosome [22].

The fate of parental imprints in mutant mice with impaired methylation is striking: the imprints on three genes including *Igf2* and *H19* are lost, resulting in either repression (*Igf2*) or expression of both parental copies (H19) and the death of mutant embryos. In agreement with previous proposals, the paternal *Igf2/H19* domain becomes functionally maternal [23]. This confirms the view that DNA methylation is a key event in the imprinting process. Embryos with the maternal disomy for chromosome 7 have excess *H19* and no *Igf2* (just like the methyltransferase mutant embryos) and are lethal [7]. However, the observation that both alleles of the two genes are expressed in androgenetic mononuclear trophoblasts also suggests that a biparental contribution may be required for expression of the reciprocal *Igf2/H19* imprint. In addition, that imprinted regions replicate asynchromously and that, whatever the imprinting pattern, it is the paternal chromosome which replicates earlier, adds further complexity to this puzzling syndrome [8]. However, as seen for the Prader-Will/Angelman region, the asynchronous replication pattern may be more complex.

The regulation of imprinting is thus a complex process that utilizes multiple control mechanisms and most certainly involves local and regional effectors of expression.

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