Review

Genomic Imprinting: Mechanism and Role in Human Pathology

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Most genes are expressed from two alleles, one maternal and the other paternal. The term "genomic imprinting" refers to a genetic phenomenon which produces some interesting exceptions to this rule. Genes which are subject to imprinting are molecularly marked before fertilization such that they are transcriptionally silenced at one of the parental alleles in the offspring. A growing body of evidence implicates genomic imprinting in the pathogenesis of certain human genetic diseases, inherited tumor syndromes, and sporadic tumors. This review discusses examples of imprinting, theories as to why the phenomenon exists, possible molecular mechanisms of imprinting, and our current understanding of the role of imprinting in human pathology. (Am J Pathol 1994, 144:431–443)

Genomic Imprinting in Mice and Humans

Among the first and most general indications of the existence of genomic imprinting in mammals were observations of aberrant development of embryos after experimental induction of parthenogenesis in mice.[1-3] True parthenotes, induced by ethanol exposure of oocytes or gynogenones constructed by replacement of male pronuclei with female pronuclei, were found to grow to early somite stages before involution. Up to this stage, these conceptuses were relatively normal in size and appearance, but they showed unusually small extraembryonic membranes. An inverse situation was seen in androgenones induced by transplantation of male pronuclei into ova in which the female pronucleus had been removed.

These developed to the late preimplantation stage but often failed to implant; the small percentage which were able to implant gave rise to predominantly extraembryonic placental tissues, with severely stunted embryonic tissues. From these experiments it was concluded that maternal and paternal genomes are both essential for the development of mice past early embryonic stages and that their contributions to growth of the early conceptus are not equivalent. In particular, it was proposed that certain genes which are essential for growth of trophoblastic tissue are expressed preferentially or exclusively from the paternally transmitted genome, while the maternally transmitted genome can provide all of the essential gene activities needed for early development of the tissues of the embryo proper but lacks essential activities for growth of the trophoblast. This idea was borne out by cytogenetic studies of human hydatidiform moles and benign ovarian teratomas or dermoid cysts. Moles, which are composed mostly of trophoblastic tissue, were found to contain a reduplicated paternal complement of chromosomes,[4] while dermoids, which differentiate into a broad spectrum of somatic tissues but which never show placental elements, invariably contained a reduplicated complement of maternal chromosomes derived from an unfertilized oocyte.[5] Consistent with this, teratomas and teratocarcinomas can be produced in mouse ovaries by inducing ova to undergo parthenogenesis *in situ*.[6] In addition to supporting the existence of non-overlapping sets of paternally and maternally imprinted genes, these findings gave the first hint of a possible role for genomic imprinting in tumorigenesis.

More specific evidence for the non-equivalence of maternal and paternal genomes came from breeding

experiments using lines of mice which carried various chromosomal translocations. In appropriate crosses it was possible to produce uniparental disomies for particular chromosomes or chromosomal regions.[7-10] For example, one type of cross generated fetuses containing two copies of a large portion of the maternal chromosome 7 but no copies of the corresponding portion of the paternal chromosome 7. These fetuses were developmentally retarded, showed small placentas, and died *in utero* at midgestation. The converse cross, resulting in two paternal and no maternal chromosome 7 homologues, produced conceptuses which died at a much earlier stage. By this approach, several different chromosomes and subchromosomal regions were scored for their ability to produce an abnormal phenotype when they were inherited as uniparental disomies. Since only a subset of chromosomal regions showed evidence of harboring imprinted genes by this type of assay, these studies allowed the construction of a low-resolution "imprinting map" of the mouse genome.[11] In parallel with this research in mice, evidence was being accrued by clinical geneticists indicating that certain human genetic syndromes are transmitted in a pattern consistent with parental imprinting and/or are associated with uniparental disomies or parent-of-origin-specific aberrations of certain chromosomal regions. Chromosomal mapping of these imprinted disease loci has suggested that for at least some syntenic chromosomal regions there might be good agreement between the mouse and human "imprinting maps."[10,11]

Historically, however, it was a series of reports in the late 1980s showing that in certain lines of transgenic mice the transgene is subject to allele-specific DNA methylation, and in some cases allele-restricted mRNA expression, which brought the imprinting phenomenon into focus for the general community of molecular biologists.[12-15] In fact, imprinting observed at transgenic loci may be somewhat artificial because of the potential for unpredictable effects of the chromosomal site of insertion on subsequent gene expression and/or because the microinjected transgene DNA may be extensively modified by the zygotic DNA methyltransferase before or after its insertion into the chromosome. Nevertheless, the expectation that certain endogenous genes, residing in their normal chromosomal locations, would eventually be found to be imprinted was fulfilled a few years later when breeding experiments utilizing either artificially constructed or naturally occurring strains of mice in which the two types of parental alleles could be conveniently distinguished showed that the insulin-like growth factor 2 (Igf2) gene was expressed as mRNA only from the paternal allele and that the genes encoding the Igf2 receptor (Igf2R) and a differentiation-related fetal RNA (H19) were expressed only from the maternal alleles.[16-18] Two of these genes, Igf2 and H19, were subsequently shown to be monoallelically expressed in humans, with evolutionary conservation of the parental "direction" of the imprinting in both cases.[19-24]

Evolutionary Rationale for Imprinting

The evolutionary conservation of imprinting between mice and humans suggests that the phenomenon may provide some selective advantage. Several theoretical advantages of imprinting for the success of the species have been proposed, all of which are at this point completely speculative. By rendering parthenotes nonviable, it has been suggested that imprinting might function to ensure that the species continued to propagate by sexual reproduction.[25] However, certain non-mammalian species which can produce viable parthenotes and which may lack genomic imprinting still seem to prefer to propagate sexually. A different theory, which views imprinting as a form of fetal-maternal or paternal-maternal competition, has been suggested based on the observations in parthenotes and uniparental disomies.[26,27] According to this scheme, paternally imprinted genes have a negative effect on growth of the placenta and fetus, while the converse is true for maternally imprinted genes. It is argued that the male parent will achieve more success in perpetuating its genome within the population if it can promote large placentas and large offspring, while the female parent will achieve more evolutionary success by limiting the size of the placenta and offspring so that the nutritional burden of each pregnancy is reduced and successive pregnancies can be sustained.

A third possible rationale for the existence of imprinting, which has not been raised previously, is that the phenomenon might serve to maintain diploidy in dividing cells. Because of potential deleterious effects of chromosomal losses, primarily the predicted tendency of monosomies to predispose cells to oncogenic transformation by loss of tumor suppressor genes, it might be advantageous for the species to have a built-in safeguard against such losses. If a particular chromosome were to contain two distinct growth-essential genes which were oppositely imprinted, then cell clones which had suffered a loss of either homologue of this chromosome (in mitotic errors such as chromosomal nondisjunction) would be prevented from expanding. In this way, the species might be protected

from the occurrence of certain malignant tumors or other adverse consequences of chromosomal monosomies.

It is also possible that imprinting *per se* might not provide an advantage to the species and instead might simply be a byproduct of other evolutionary pressures. A theory which invokes defense against viral pathogens as a selective pressure[28] stems from observations that invading viruses can be inactivated by the host cell via methylation of CpG dinucleotides in the viral genome.[29] Since CpG methylation is a strong candidate for the molecular modification underlying imprinting (see below), this theory suggests that the imprinting phenomenon might simply be a byproduct of the evolutionary pressure to maintain this pathway of host defense. In other words, imprinted genes might be innocent bystanders which are recognized by the methylation machinery of one type of gamete as virus-like because of some feature of their nucleotide sequence. Reasoning along these same lines, the CpG methylase may well be essential in general gene regulation, where it appears to consolidate the transcriptionally repressed state of some tissue-specific genes in non-expressing cell types and where it seems to play a similar role in the process of X chromosome inactivation.[30,31] In the most trivial model, conserved sequence features of certain genes which merely reflect the functional requirements of their promoter or coding regions might cause these genes to be acted upon by the methyltransferase in one or the other type of germline in a pattern which would coincidentally result in their becoming imprinted.

For those interested in the *raison d'etre* of imprinting, the most important unanswered question concerns the size and characteristics of the imprinted fraction of the genome. In terms of an upper limit, the fact that most genetic diseases show Mendelian inheritance implies that most genes will turn out not to be imprinted. In terms of a lower limit, the experiments with uniparental disomies in mice have suggested the existence of at least six distinct imprinted regions on the 19 autosomes.[9-11] Progress in defining the size of the set of genes which are subject to imprinting, and the shared characteristics, if any, of the genes in this set, awaits the development of efficient screening methods for identifying and cloning imprinted genes.

Mechanism of Imprinting

Any biochemical modification of the DNA and/or chromatin which can account for imprinting must satisfy four requirements. First, the modification must be made before fertilization. Second, it must be able to confer transcriptional silencing. Third, it must be stably transmitted through mitosis in somatic cells. Fourth, it must be reversible on passage through the opposite parental germline. Some fairly exotic possibilities are consistent with these requirements. For example, imprinted genes could conceivably contain DNA sequences which undergo a reversible physical rearrangement, such as a precise inversion, during oogenesis and spermatogenesis. A second possibility is that imprinted genes could interact with hypothetical oocyte or spermatocyte-specific DNA binding proteins which could establish tenacious complexes capable of persistence and replication in zygotic cell divisions but also susceptible to displacement during the next cycle of gametogenesis. While these models may have precedents in lower organisms, they have yet to receive any experimental support in mammalian systems. In contrast, a third model, which proposes site-specific DNA methylation as the imprinting mechanism, is supported both by *a priori* considerations and by a growing body of experimental evidence.

Methylation of DNA in mammalian cells occurs exclusively at cytosine residues in the context of CpG dinucleotides. CpG methylation of genes, particularly in their promoter regions, can render them transcriptionally silent, and CpG methylation is faithfully transmitted through cell divisions by the action of the maintenance DNA methyltransferase (reviewed in refs. 32–34). While the modification is stably propagated in the presence of an active methyltransferase, it can be reversed when DNA replicates under conditions in which the methyltransferase is inhibited or sequestered, as may be the case in very early development.[35] In fact, the erasure of methylation of many DNA sequences in the immediate post-zygotic period and during the formation of the germ cells is well documented.[33,36-41] In terms of the mature gametes, for several different types of sequences, the DNA in sperm is known to be CpG-methylated in a different pattern from the DNA in ova.[33,36-38,40,41] While much of this difference appears to be erased in somatic cells in early development, allelic methylation differences might well persist at certain demethylation-resistant sites in the genome.

The simplest methylation model for imprinting posits that, because of a critical positioning and/or density of CpGs found uniquely at imprinted genes (or gene clusters, see below), gametic methylation differences are preserved at demethylation-resistant

sites in or near these genes in early somatic development and that these critical sites nucleate the spread of methylation and chromatin condensation during later development, resulting in allele-restricted gene expression. The local spreading of chromatin inactivation which is hypothesized in this model is suggested by the well-established spreading tendency of X chromosome inactivation[30] and by observations of progressive expansion of preimposed CpG methylation within proviral DNAs with passaging of cells in culture.[42] Attempts to define the critical DNA regions postulated to control imprinting in this and other models using transgenic mice are in progress.[43,44]

In the context of this model it is reasonable to ask whether large groups of linked genes might become coordinately imprinted. While this notion of regional imprinting is attractive in principle, the small amount of evidence available can be viewed as arguing either for or against this possibility. For example, the Igf2 and H19 genes are closely linked (within 90–200 kb) in both mice and humans, yet they are imprinted in opposite directions[45] (see also ref. 44 for an enhancer-competition model for opposite imprinting of these two loci). In addition, the ribonucleotide reductase M1 subunit gene (RRM1), located in the same chromosomal band as human IGF2 and H19, has been shown not to be imprinted[46] and several genes near Igf2R on mouse chromosome 17 are biallelically expressed.[17] On the other hand, recent studies suggest that a different pair of linked genes in the mid-portion of mouse chromosome 7, Snrpn and Znf127, may show coordinate methylation imprinting, with predicted expression of only the paternal alleles in both cases.[47,48] It may be that imprinting can spread regionally along the chromosome but that many interspersed genes somehow escape transcriptional inactivation. A situation of this sort would be analogous to that observed on the human inactive X chromosome, where an increasing number of genes are being identified which either partially or completely escape inactivation.[30,31]

Consistent with the methylation hypothesis, for every imprinted transgene and for the few endogenous imprinted loci which have been examined, clear patterns of allele-specific DNA methylation have been found. In the case of the H19 gene, allele-specific DNA methylation has been documented in fetal and adult tissues both mice and humans, with hypermethylation of the imprinted allele through the entire extent of the gene in both species.[22,44,49,50] In humans, the H19 gene in sperm is extensively methylated, while in gynogenetic ovarian teratomas the gene is largely unmethylated.[22] Relative hypermethylation of the H19 gene in sperm of mice is also detectable.[49,50] Moreover, partial methylation of the H19 promoter inhibits its ability to activate transcription of a reporter gene in transfection experiments[22] and demethylation of imprinted H19 alleles by exposure of cells to the DNA methyltransferase inhibitor 5-azacytidine (AzaC) can reactivate transcription from these alleles (T. Moulton and B. Tycko, unpublished observations). Allele-specific methylation is also present at several CpGs in the Igf2[49] and Igf2R[51] genes of mice but, in contrast to H19, the active Igf2 allele was found to be hypermethylated at the sites examined. However, the inactive Igf2 allele may be hypermethylated at regulatory sites which were not examined since, as with H19, imprinting of Igf2 could be erased by AzaC.[52] Also consistent with an important role for methylation in imprinting, it was recently reported that imprinting of H19, Igf2, and Igf2R is disrupted in mouse embryos with a targeted deletion of the DNA methyltransferase gene.[53] However, since the observed effects on imprinting in these mice might be indirect, even this impressive experiment does not prove a primary role for methylation in imprinting. Indeed, because of the potential for indirect effects and because of difficulties in definitively separating cause from effect in relating CpG methylation to the transcriptional activity of specific genes, a definitive proof of the methylation model for imprinting may be difficult to obtain.

Before leaving the topic of mechanism, of potential importance for understanding the effects of imprinting in disease states are several observations which suggest that maintenance of imprinting in somatic tissues is not always completely efficient. First, imprinting can be tissue-specific: the maternal allele of the murine Igf2 gene is silent in all tissues except choroid plexus and meninges, where the imprint is apparently not present and the maternal copy is expressed.[16] Similarly, paternal imprinting of human H19 may be at least partially relaxed in trophoblast of hydatidiform moles[20,54] and in normal placenta as well.[19] There is also evidence for at least partial relaxation of imprinting of human IGF2 and, more rarely, H19, in malignant tumor cells, perhaps secondary to alterations of DNA methylation in these cells.[21,55] Second, the efficiency of imprinting depends on genetic background: in transgenic mice the presence or absence of methylation imprinting of the transgene can be strain-dependent, suggesting the existence of imprinting modifier genes.[56–58] Also, imprinting of the Tme trait in mice may be under the control of a strain-specific modi-

fier locus,[59] although altered imprinting of a specific gene was not proven in this case. Genetic background may also effect the efficiency of imprinting in humans, as suggested by the finding of somatic reversal of imprinting of the human H19 gene in lung and cerebellum of one out of six individuals examined.[22] Whether imprinting modifier genes include the genes encoding DNA methyltransferase or a putative DNA demethylase enzyme[60] and/or genes which might indirectly regulate either methyltransferase or demethylase activity is an obvious but as yet unanswered question.

Imprinting in Human Genetic Disease

As indicated in a previous comprehensive review, at least 10 distinct human genetic diseases and syndromes have been suspected to involve genomic imprinting.[61] In some cases, the evidence for imprinting is that the trait is observed at equal frequencies in males and females but is transmitted exclusively or preferentially from one type of parent. A variation on this pattern are diseases in which both types of parents can transmit the phenotype, but a particularly severe form of the disease results from transmission by one type of parent. In several disorders which show this type of pattern, the percentage of individuals who show the predicted parental effects is lower than would be expected for a perfectly efficient process: this may reflect random fluctuations in the efficiency of imprinting and/or genetic background effects.[61] A second type of evidence for imprinting is that a disease phenotype can be found recurrently associated with uniparental disomies for a particular chromosome or chromosomal region, as revealed by comparison of DNAs from patients and their parents at polymorphic marker loci.

Among the clearest and best studied examples of imprinted genetic diseases are Prader-Willi syndrome (PWS) and Angelman syndrome (AS). Both syndromes include mental retardation (mild to moderate in PWS and severe in AS), but the associated stigmata are entirely distinct and even to some degree opposite: individuals with PWS are slow moving and become overweight due to severe hyperphagia; individuals with AS are thin and hyperactive and have a characteristic "happy puppet" appearance, with inappropriate laughter. In the late 1980s several laboratories made the observation that both syndromes often result from chromosomal deletions in bands 15q11–13 and that the deletions in the two syndromes were cytogenetically and, at that time,

molecularly indistinguishable.[62–64] A possible role for genomic imprinting in producing the distinct phenotypes was raised when it was found that the deleted DNA in the two syndromes was of opposite parental origin: in each case of PWS the deletion had occurred on the paternal chromosome 15, while for each case of AS it had occurred on the maternal homologue.[63–66] Additional evidence for opposite imprinting in the two syndromes was the finding that cases of PWS with maternal disomy for the entire chromosome 15 are fairly frequent[67] and that rare cases of AS can be caused by paternal disomy of this same chromosome.[68] Moreover, one family has been described in which inheritance of a 15q11–13 deletion apparently produced a case of AS after transmission from a mother and two cases of PWS after transmission from a father.[69] One hypothesis which emerged is that a single PWS/AS gene, imprinted paternally in some cell types and maternally in others, might account for both syndromes. In an alternative scheme, which appears to be supported by recent high-resolution mapping of the minimal deleted regions, PWS and AS are caused by two very closely linked but distinct genes (or gene clusters) which are oppositely imprinted.[70] Resolution of the issue awaits the cloning and characterization of candidate genes in the PWS and AS minimal deleted regions. Two such genes in the PWS minimal deleted region, SNRPN, encoding a polypeptide component (SmN) of a ribonucleoprotein thought to be involved in brain-specific mRNA splicing reactions, and ZNF127, encoding a putative nucleic acid binding protein, have recently been identified.[10,47,48,71]

A second fascinating and clinically important group of human diseases which have been considered to show imprinting effects are the so-called "triplet-repeat diseases." These inherited disorders, including fragile X mental retardation (FRAX), myotonic dystrophy (DM), and Huntington's disease (HD), among others, result from the presence of repetitive trinucleotide DNA sequences in the disease genes (reviewed in ref. 72). In FRAX and probably also in DM, it appears that when the repeated sequences become longer than a critical length, they become unstable and can undergo massive length expansions during cellular DNA replication, thereby functionally inactivating their associated genes and causing the disease phenotype. The initial moderate expansion, which exists in asymptomatic disease carriers and which renders the locus unstable, has been called the "premutation," while the massive expansion in affected individuals is referred to as the "full mutation."[73] While the repeats in HD are

not subject to such massive expansions, premutation alleles may also exist.[74]

Imprinting in each of these triplet repeat diseases has been suggested by clinical observations: the disease phenotypes show earlier onset (DM, HD) or greater severity (FRAX) in the offspring after passage through the germline of one type of parent—mothers in FRAX and DM and fathers in HD.[75–77] In the case of DM, only affected mothers can transmit a distinctive severe congenital form of the disease. However, since congenitally affected infants who survive the neonatal period tend to recover from their severe symptoms, it may be that some physiological influence of the uterine environment, rather than true genomic imprinting, is responsible for the selective maternal "transmission." In the case of FRAX, the evidence for imprinting is stronger. The basic observation implicating imprinting is that some males can be phenotypically normal carriers of the premutation and that successive passages of the premutated gene through females convey an increasing likelihood of appearance of an affected son with the full mutation.

Even with a knowledge of the unusual molecular behavior of triplet repeats, an explanation for the parental imprinting has not been immediately apparent. In HD a general but imperfect correlation between greater repeat lengths and earlier onset disease has been found (reviewed in ref. 78) and the accelerated onset after paternal transmission may reflect a greater tendency for repeat expansion in spermatogenesis *versus* oogenesis. Strictly speaking, this would then be a genetic effect distinct from true imprinting. However, this trivial explanation does not apply in FRAX, where the expansions to the full mutation have been shown to be post-zygotic events.[79,80] Since the FRAX triplet repeat contains CpG and is highly methylated only in the expanded alleles, maternal-specific hypermethylation of the premutation, perhaps as a consequence of X-inactivation, could play a role in marking it for subsequent expansion.[75,81–84] Whether or not this explanation proves to be correct, since some type of maternal imprinting appears to be intimately related to the propensity of the FRAX triplet repeat to expand, an understanding of the molecular features of the imprint may also shed light on the mechanism of triplet repeat instability.

Imprinting in Tumorigenesis

As is the case for the classical human genetic diseases, the evidence for imprinting in human tumorigenesis takes several forms. In at least one familial

tumor syndrome, the inherited paraganglioma syndrome, the phenotype, usually bilateral carotid body tumors, is only manifested after transmission of the disease gene from fathers.[85] Since the high frequency of affected individuals in the pedigrees is otherwise consistent with autosomal dominant transmission, it has been predicted that the chromosome 11q23-qter gene accounting for this syndrome will turn out to be a maternally imprinted/paternally expressed dominant oncogene.[86]

The retinoblastoma (RB) gene is well known as the prototype tumor suppressor gene predicted in the classical "two-hit" model for recessive oncogenesis.[87,88] Perhaps surprisingly in view of the demonstrated necessity for biallelic inactivation of RB in the development of retinoblastomas, there are also hints that the RB locus might be subject to genomic imprinting. Evidence for imprinting of RB has come not from studies of retinoblastomas but instead from observations in a different type of RB-related tumor, sporadic osteosarcoma. In these tumors there is a strong bias in the parental origin of RB allele losses. In one study, 90% of cases showed loss of the maternal RB allele, presumably with mutation at the retained paternal allele.[89] Since the tumors examined were of relatively late onset and were not preceded by retinoblastomas, they probably contained somatic rather than germline RB mutations. From this it was concluded that, rather than resulting from a parental bias in the germline mutation rate, the observed bias in RB allele losses probably reflects *bona fide* genomic imprinting. While evidence for differences in DNA methylation at maternal *versus* paternal RB alleles in leukocytes and fibroblasts has been reported,[90] whether there are allelic differences in the level of RB mRNA expression in osteosarcoma precursor cells or other cell types is not yet known. If an allelic bias exists, it may be restricted to those rare individuals who subsequently develop osteosarcoma and indeed may predispose them to this tumor by allowing the first genetic "hit" of the RB gene, when it occurs on the more highly expressed allele, to partially release the cell from normal growth regulation. Observations that about 5 to 10% percent of unilateral retinoblastomas show hypermethylation of the RB promoter and first exon and that the hypermethylation can be allele-restricted give some support to these speculations.[91,92]

More recently, evidence has been produced suggesting parental imprinting of both a dominant oncogene and a putative tumor suppressor gene involved in human neuroblastoma. Amplification of a large segment of DNA containing the N-*myc* proto-

oncogene is a frequent finding in neuroblastomas, where the presence of amplification confers a poor prognosis.[93,94] When the parental origin of the amplified DNA was examined using polymorphic DNA markers, 12 of 13 cases showed amplification of the paternal N-*myc* allele.[95] Whether this parental bias reflects an allelic bias in N-*myc* mRNA expression in neuroblasts remains to be determined. Neuroblastomas also frequently show loss of DNA in chromosomal band 1p36, implicating a putative tumor suppressor gene in this region. In one study[96] the lost 1p36 DNA was found to be selectively of maternal origin (13 of 15 cases), but this bias was not found in a second study.[95]

A role for genomic imprinting in tumorigenesis is perhaps most firmly established by findings in the Beckwith-Wiedemann syndrome (BWS) and in a group of embryonal tumors which are associated with this syndrome. BWS is diagnosed by the presence of variable somatic manifestations, including exomphalos, macroglossia, visceromegaly (including organomegaly affecting kidney, liver, and adrenal), hemihypertrophy, and gigantism, all of which reflect overgrowth of developing tissues, and a high percentage of affected individuals will develop Wilms' tumor (WT), adrenocortical carcinoma (ADCC), hepatoblastoma (HB), or embryonal rhabdomyosarcoma (ER)[97] (reviewed in ref. 98). The evidence for imprinting in BWS takes several forms. In some families, the trait is associated with constitutional chromosomal inversions or translocations at 11p15.4 or 11p15.5, but the phenotype is only expressed after passage of the structurally abnormal chromosome through the maternal germline.[98,99] The syndrome can also be transmitted within families with no cytogenetic abnormalities but with genetic linkage to chromosome 11p15; here also, the phenotypic is only seen after passage of the disease gene through the maternal germline.[100-103] Perhaps more frequently, the syndrome can occur *de novo* in association with paternal duplication or isodisomy for 11p15.5.[104-107] In fact, mice which are constructed as genetic mosaics for paternal disomy of the homologous chromosomal region are a potential animal model for BWS and show increased body size.[108] Last but not least, numerous studies indicate that each of the four types of embryonal tumors which are associated with BWS show frequent loss of heterozygosity (LOH) for DNA markers at 11p15.5 (reviewed in refs. 98 and 109) and, importantly, in series of WTs and ERs (consisting primarily of sporadic rather than BWS-associated cases), there is a very strong (95–100%) bias toward loss of maternal 11p15.5 alleles.[110-113]

Can these observations be reconciled in a comprehensive theory of the role of genomic imprinting in the etiology of BWS and embryonal tumors? Most of the observations are consistent with the hypothesis that BWS is caused by abnormal expression of one particular imprinted gene, IGF2. As mentioned previously, this gene maps to 11p15.5 and is normally expressed only from the paternal allele. According to the "IGF2 hypothesis," paternal disomies or duplications of chromosome 11p15.5 would be expected to lead to a twofold increase in Igf-2 protein production and a corresponding increased growth of Igf2-responsive tissues, accounting for the characteristic organomegaly of BWS. The chromosomal rearrangements, deletions, or putative point mutations in the 11p15.4–15.5 region which account for the remaining cases of BWS, all of which show selective maternal transmission, are postulated to somehow cause a failure of imprinting of the maternal IGF2 allele, thereby leading to the same endpoint of increased Igf-2 protein production.[98,99,114] This aspect of the hypothesis was recently confirmed by the finding of biallelic IGF2 expression in fibroblasts and tongue tissue of BWS patients who lacked paternal 11p15 disomies.[114] Since structural lesions of DNA sequences within the IGF2 gene were not found in any of the cases, the disruption of imprinting may be a long-range chromosomal effect.

If one accepts IGF2 as the BWS gene, then the remaining question is whether overexpression of Igf-2 protein could account not only for tissue overgrowth but for tumor formation as well. The finding of recurrent LOH in a particular chromosomal region in tumors is usually taken as evidence for the existence of a tumor suppressor gene in that location. By this simple interpretation, the finding of selective loss of maternal 11p15.5 alleles in WT and ER implies the existence of a paternally imprinted embryonal tumor suppressor gene at 11p15.5. Based on its required direction of imprinting and expected biological function (growth-inhibiting rather than growth-promoting), this gene must be distinct from IGF2.

However, before considering evidence for the existence of such a gene, it is important to note that most WTs and ERs which have lost maternal alleles at 11p15.5 are also found to contain duplicated paternal alleles. Presumably this results in two active copies of IGF2 per cell. It has been proposed that this paternal duplication, rather than the maternal loss, may be the etiologically important event.[114-116] However, while a twofold increase in active IGF2 allele copy number may well be respon-

sible for the tissue overgrowth seen in somatic tissues in BWS, whether it produces functionally important differences in Igf-2 protein production in the BWS-associated tumors is less clear. In two large studies nearly all WTs, presumably regardless of the presence or absence of 11p15.5 LOH, were found to express high levels of IGF2 mRNA, comparable with the levels in fetal kidney.[117,118] Given the ability of transcription factors to modulate promoter activities over a range of several orders of magnitude, this high level expression probably reflects the presence in the tumor cells of the appropriate array of transcription factors for maximal activation of the IGF2 promoter, rather than a gene dosage effect. Interestingly, despite the high levels of IGF2 mRNA, the amount of immunoreactive Igf-2 protein in WTs appears to be quite low.[119,120]

The identity of the putative 11p15.5 embryonal tumor suppressor gene, sometimes referred to as "WT2" (to distinguish it from the previously identified WT1 tumor suppressor gene at 11p13), is a major unresolved issue. What criteria can be applied to evaluate candidate WT2 genes? Based on the observed selective loss of maternal 11p15.5 alleles in embryonal tumors, we have previously suggested that a criterion for candidate genes is that they should be expressed only from the maternal allele in normal fetal tissues.[19,22] A tumor suppressor gene which was expressed monoallelically in normal tissues would be expected to represent an "Achilles' heel" for cellular transformation, since complete functional inactivation of this gene could occur by "one-hit" kinetics. Rapid kinetics of inactivation could account for the fact that some WTs, ERs, HBs, and ADCCs are seen in newborns and, particularly in the case of WT, often present as bilateral or multifocal lesions. However, this may be an oversimplification. Different schemes can be envisioned in which paternal imprinting of the WT2 locus might occur only in those rare individuals who subsequently develop tumors[58,121] or in which the WT2 tumor suppressor is not imprinted and the observed selective loss of maternal alleles is due entirely to a selective pressure to retain the active paternal IGF2 allele.[115,116]

One 11p15.5 gene which is paternally imprinted is already available for evaluation as a candidate WT2 gene. The human H19 gene is expressed exclusively from the maternal allele.[19,21,22] Consistent with a growth-regulatory role, expression of this gene is very low in undifferentiated cells, increases markedly in a wide array of fetal tissues at stages in which cells are differentiating, and then declines in most adult tissues.[122–126] In fact, H19 was isolated

independently by several different laboratories as a differentiation-induced clone in differential cDNA screening experiments using various cell culture systems.[123,126,127] Also, as might be expected for a gene involved in cellular differentiation and growth suppression, transcription of H19 is very high in normal fetal kidney, adrenal, and liver but is very low or undetectable in a majority of WTs and in at least some cases of ADCC and HB (T. Moulton et al, submitted for publication).

H19 is transcribed to yield a spliced and polyadenylated RNA which accumulates in the cytoplasm but which contains only very short translational reading frames.[128] Moreover, while there is overall conservation of intron/exon structure and nucleotide sequence between H19 genes of human, mouse, and rat, the short reading frames are not conserved. Based on these findings, together with the failure to detect H19-derived peptides using antipeptide antibodies, it has been proposed that H19 might function directly at the level of its RNA product, perhaps as the RNA component of a ribonucleoprotein.[128]

To date there are two lines of evidence which suggest that H19 RNA has growth-regulatory activity. First, H19 transgenic mice which expressed the transgene at high levels and in ectopic sites died *in utero* at a late fetal stage; only when the transgene was internally deleted could viable offspring be obtained.[129] Second, when an expression vector containing the human H19 gene was introduced into G401 cells, a line derived from a WT or a malignant rhabdoid tumor of the kidney,[130,131] the cells expressed high levels of H19 RNA and became nonclonogenic in soft agar and non-tumorigenic in nude mice.[132] In addition, transfection of this same expression construct into an ER cell line yielded a high percentage of growth-retarded clones.[132] Definitive evidence that H19, or other candidate tumor suppressor genes, are in fact WT2 will have to include the identification in tumors of DNA lesions such as mutations, small deletions, or perhaps even localized LOH within or very close to the candidate gene.

Future Research

To understand the biological rationale and consequences of imprinting, it will first be necessary to define the identities and functions of what must be a fairly large number of as yet uncharacterized imprinted genes. While some of these, such as the PWS and AS genes, will no doubt be identified in

the near future, innovative strategies will be required to carry out a more general search. In terms of the mechanism of imprinting, new insights may come from the study of the control of CpG methylation and demethylation in early development and from the cloning and characterization of imprinting modifier genes. Finally, a more complete understanding of the role of imprinting in neoplasia can be expected to emerge rapidly from the current intense scrutiny of the molecular pathology of human embryonal tumors.

Acknowledgments

References

1. Surani MAH, Barton SC, Norris ML: Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. Nature 1984, 308:548–550
2. Barton SC, Surani MAH, Norris ML: Role of paternal and maternal genomes in mouse development. Nature 1984, 311:374–376
3. McGrath J, Solter D: Completion of mouse embryogenesis requires both the maternal and paternal genomes. Cell 1984, 37:179–183
4. Lawler SD, Povey S, Fisher RA, Pickthal VJ: Genetic studies on hydatidiform moles. II. The origin of complete moles. Ann Hum Genet 1982, 46:209–222
5. Linder D, McCaw BK, Hecht F: Parthenogenic origin of benign ovarian teratomas. N Engl J Med 1975, 292:63–66
6. Stevens LC, Varnum DS: The development of teratomas from parthenogenetically activated ovarian mouse eggs. Dev Biol 1974, 37:369–380
7. Searle AG, Beechey CV: Complementation studies with mouse translocations. Cytogenet Cell Genet 1978, 20:282–303
8. Cattanach BM, Kirk M: Differential activity of maternally and paternally derived chromosome regions in mice. Nature 1985, 315:496–498
9. Searle AG, Beechey CV: Genome imprinting phenomena on mouse chromosome 7. Genet Res 1990, 56:237–244
10. Cattanach BM, Barr JA, Evans EP, Burtenshaw M, Beechey CV, Leff SE, Brannan CI, Copeland NG, Jenkins NA, Jones J: A candidate mouse model for Prader-Willi syndrome which shows an absence of Snrpn expression. Nature Genet 1992, 2:270–274
11. Searle AG, Peters J, Lyon MF, Hall JG, Evans EP, Edwards JH, Buckle VJ: Chromosome maps of man and mouse IV. Ann Hum Genet 1989, 53:89–140
12. Hadchouel M, Farza H, Simon D, Tiollais P, Pourcel C: Maternal inhibition of hepatitis B surface antigen gene expression in transgenic mice correlates with de novo methylation. Nature 1987, 329:454–456
13. Reik W, Collick A, Norris ML, Barton SC, Surani, MA: Genomic imprinting determines methylation of parental alleles in transgenic mice. Nature 1987, 328:248–251
14. Sapienza C, Peterson AC, Rossant J, Balling R: Degree of methylation of transgenes is dependent on gamete of origin. Nature 1987, 328:251–254
15. Swain JL, Stewart TA, Leder P: Parental legacy determines methylation and expression of an autosomal transgene: a molecular mechanism for parental imprinting. Cell 1987, 50:719–727
16. DeChiara TM, Robertson EJ, Efstratiadis A: Paternal imprinting of the mouse insulin-like growth factor II gene. Cell 1991, 64:849–859
17. Barlow DP, Stoger R, Hermann BG, Saito K, Schweifer N: The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. Nature 1991, 349:84–87
18. Bartolomei MS, Zemel S, Tilghman SM: Parental imprinting of the mouse H19 gene. Nature 1991, 351:153–155
19. Zhang Y, Tycko, B: Monoallelic expression of the human H19 gene. Nature Genet 1992, 1:40–44
20. Rachmilewitz J, Goshen R, Ariel I, Schneider T, de Groot N, Hochberg A: Parental imprinting of the human H19 gene. FEBS Lett 1992, 309:25–28
21. Rainier S, Johnson L, Dobry CJ, Ping AJ, Grundy PE, Feinberg AP: Relaxation of imprinted genes in human cancer. Nature 1993, 362:747–749
22. Zhang Y, Shields T, Crenshaw T, Hao Y, Moulton T, Tycko B: Imprinting of human H19: allele-specific CpG methylation, loss of the active allele in Wilms' tumor and potential for somatic allele switching. Am J Hum Genet 1993, 53:113–124
23. Ohlsson R, Nystrom A, Pfeifer-Ohlsson S, Tohonen V, Hedbourg F, Schofield P, Flam F, Ekstrom TJ: IGF2 is parentally imprinted during human embryogenesis and in the Beckwith-Wiedemann syndrome. Nature Genet 1993, 4:94–97
24. Giannoukakis N, Deal C, Paquette J, Goodyer CG, Polychronakos C: Parental genomic imprinting of the human IGF2 gene. Nature Genet 1993, 4:98–101
25. Solter D: Inertia of the embryonic genome in mammals. Trends Genet 1987, 3:23–27
26. Haig D, Graham C: Genomic imprinting and the strange case of the insulin-like growth factor II receptor. Cell 1991, 64:1045–1046
27. Moore T, Haig D: Genomic imprinting in mammalian development: a parental tug of war. Trends Genet 1991, 7:45–49

28. Barlow DP: Methylation and imprinting: from host defense to gene regulation? Science 1993, 260:309–310

29. Jahner D, Jaenisch R: Chromosomal position and specific demethylation in enhancer sequences of germline-transmitted retroviral genomes during mouse development. Mol Cell Biol 1985, 5:2212–2220

30. Lyon MF: Some milestones in the history of X-chromosome inactivation. Annu Rev Genet 1992, 26:17–28

31. Riggs AD, Pfeifer, GP: X-chromosome inactivation and cell memory. Trends Genet 1992, 8:169–174

32. Dynan WS: Understanding the molecular mechanism by which methylation influences gene expression. Trends Genet 5:35–36

33. Cedar H, Razin A: DNA methylation and development. Biochim Biophys Acta 1990, 1049:1–8

34. Bird A: The essentials of DNA methylation. Cell 1992, 70:5–8

35. Carlson LL, Page AW, Bestor TH: Properties and localization of DNA methyltransferase in preimplantation mouse embryos: implications for genomic imprinting. Genes Dev 1992, 6:2536–2541

36. Monk M, Boubelik M, Lehnert S: Temporal and regional changes in DNA methylation in the embryonic, extraembryonic, and germ cell lineages during mouse development. Development 1987, 99:371–382

37. Sanford JP, Clark HJ, Chapman VM, Rossant J: Differences in DNA methylation during oogenesis and spermatogenesis and their persistence during early embryogenesis in the mouse. Genes Dev 1987, 1:1039–1046

38. Driscoll DJ, Migeon BR: Sex difference in methylation of single-copy genes in human meiotic germ cells: implications for X chromosome inactivation, parental imprinting, and origin of CpG mutations. Somat Cell Mol Genet 1990, 16:267–282

39. Frank D, Keshet I, Shani M, Levine A, Razin A, Cedar H: Demethylation of CpG islands in embryonic cells. Nature 1991, 351:239–241

40. Howlett SK, Reik W: Methylation levels of maternal and paternal genomes during preimplantation development. Development 1991, 113:119–127

41. Kafri T, Ariel M, Brandeis M, Shemer R, Urven L, McCarrey J, Cedar H, Razin A: Developmental pattern of gene-specific DNA methylation in the mouse embryo and germ line. Genes Dev 1992, 6:705–714

42. Toth M, Lichtenberg U, Doerfler, W: Genomic sequencing reveals a 5-methylcytosine-free domain in active promoters and the spreading of preimposed methylation patterns. Proc Natl Acad Sci USA 1989, 86:3728–3732

43. Lee JE, Tantravahi U, Boyle AL, Efstratiatis A: Parental imprinting of an Igf-2 transgene. Mol Reprod Dev 1993, 35:382–390

44. Bartolomei MS, Webber AL, Brunkow ME, Tilghman SM: Epigenetic mechanisms underlying the imprinting of the mouse H19 gene. Genes Dev 1993, 7:1663–1673

45. Zemel S, Bartolomei SM, Tilghman SM: Physical linkage of two mammalian imprinted genes, H19 and insulin-like growth factor 2. Nature Genet 1992, 2:61–65

46. Byrne JA, Smith PJ: The 11p15.5 ribonucleotide reductase M1 subunit locus is not imprinted in Wilms' tumour and hepatoblastoma. Hum Genet 1993, 91:275–277

47. Leff SE, Brannan CI, Reed ML, Ozcelik T, Francke U, Copeland NG, Jenkins NA: Maternal imprinting of the mouse Snrpn gene and conserved linkage homology with the human Prader-Willi syndrome region. Nature Genet 1992, 2:259–264

48. Driscoll DJ, Waters MF, Williams CA, Zori RT, Glenn CC, Avidano KM, Nicholls RD: A DNA methylation imprint, determined by the sex of the parent, distinguishes the Angelman and Prader-Willi syndromes. Genomics 1992, 13:917–924

49. Brandeis M, Kafri T, Ariel M, Chaillet JR, McCarrey J, Razin A, Cedar H: The ontogeny of allele-specific methylation associated with imprinted genes in the mouse. EMBO J 1993, 12:3669–3677

50. Ferguson-Smith AC, Sasaki H, Cattanach BM, Surani MA: Parental-origin-specific epigenetic modification of the mouse H19 gene. Nature 1993, 362:751–755

51. Stoger R, Kubicka P, Liu C-G, Kafri T, Razin A, Cedar H, Barlow DP: Maternal-specific methylation of the imprinted Igf2r locus identifies the expressed locus as carrying the imprinting signal. Cell 1993, 73:61–72

52. Eversole-Cire P, Ferguson-Smith AC, Sasaki H, Brown KD, Cattanach BM, Gonzales FA, Surani MA, Jones PA: Activation of an imprinted Igf2 gene in mouse somatic cell cultures. Mol Cell Biol 1993, 13:4928–4938

53. Li E, Beard C, Jaenisch R: Role for DNA methylation in genomic imprinting. Nature 1993, 366:362–365

54. Mutter GL, Stewart CL, Chaponot ML, Pomponio RJ: Oppositely imprinted genes H19 and insulin-like growth factor 2 are coexpressed in human androgenetic trophoblast. Am J Hum Genet 1993, 53:1096–1102

55. Ogawa O, Eccles MR, Szeto J, McNoe LA, Yun K, Maw MA, Smith PJ, Reeve AE: Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms' tumour. Nature 1993, 362:749–751

56. Sapienza C, Paquette J, Tran TH, Peterson A: Epigenetic and genetic factors affect transgene methylation imprinting. Development 1989, 107:165–168

57. Allen ND, Norris ML, Surani MA: Epigenetic control of transgene expression and imprinting by genotype-specific modifiers. Cell 1990, 61:853–861

58. Sapienza C, Tran T-H, Paquette J, McGowan R, Peterson A: A methylation mosaic model for mammalian genome imprinting. Prog Nucleic Acid Res Mol Biol

1990, 36:145–157

59. Forejt J, Gregorova S: Genetic analysis of genomic imprinting: an *Imprintor-1* gene controls inactivation of the paternal copy of the mouse Tme locus. Cell 1992, 70:443–450

60. Jost J-P: Nuclear extracts of chicken embryos promote an active demethylation of DNA by excision repair of 5-methyldeoxycytidine. Proc Natl Acad Sci USA 1993, 90:4684–4688

61. Hall JG: Genomic Imprinting: Review and relevance to human diseases. Am J Hum Genet 1990, 46:857–873

62. Donlon TA: Similar molecular deletions on chromosome 15q11.2 are encountered in both the Prader-Willi and Angelman syndromes. Hum Genet 1988, 80:322–328

63. Knoll JHM, Nicholls RD, Magenis RE, Graham JM Jr, Lalande M, Latt SA: Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. Am J Med Genet 1989, 32:285–290

64. Magenis RE, Toth-Fejel S, Allen LJ, Black M, Brown M, Budden S, Cohen R, Friedman JM, Kalousek D, Zonana J, Lacy D, LaFranchi S, Lahr M, Macfarlane J, Williams CPS: Comparison of the 15q deletion in Prader-Willi and Angelman syndromes: specific regions, extent of deletions, parental origin and clinical consequences. Am J Med Genet 1990, 35:333–349

65. Butler MB, Palmer CG: Parental origin of chromosome 15 deletion in Prader-Willi syndrome. Lancet 1983, 1:1285–1286

66. Williams CA, Zori RT, Stone JW, Gray BA, Cantu ES, Ostrer H: Maternal origin of 15q1:1–13 deletions in Angelman syndrome suggests a role for genomic imprinting. Am J Med Genet 1990, 35:350–353

67. Nicholls RD, Knoll JHM, Butler MG, Karam S, Lalande M: Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome. Nature 1989, 342:281–285

68. Malcolm S, Clayton-Smith J, Nichols M, Robb S, Webb T, Armour JAL, Jeffreys AJ, Pembrey ME: Uniparental paternal disomy in Angelman's syndrome. Lancet 1991, 337:694–697

69. Hulten M, Armstrong S, Challinor P, Gould C, Hardy G, Leedham P, Lee T, McKeown C: Genomic imprinting in an Angelman and Prader-Willi translocation family. Lancet 1991, 338:638–639

70. Wagstaff J, Knoll JHM, Glatt KA, Shugart YY, Sommer A, Lalande M: Maternal but not paternal transmission of 15q1:1–13-linked nondeletion Angelman syndrome leads to phenotypic expression. Nature Genet 1992, 1:291–294

71. Ozcelik T, Leff S, Robinson W, Donlon T, Lalande M, Sanjines E, Schinzel A, Francke U: Small nuclear ribonucleoprotein polypeptide N (SNRPN), an expressed gene in the Prader-Willi syndrome critical region. Nature Genet 1992, 2:265–269

72. Mandel J-L: Questions of expansion. Nature Genet 1993, 4:8–10

73. Fu Y-H, Kuhl DPA, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, Verkek AJMH, Holden JJA, Fenwick RG Jr, Warren ST, Oostra BA, Nelson DL, Caskey CT: Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 1991, 67:1047–1058

74. Goldberg YP, Kremer B, Andrew SE, Theilmann J, Graham RK, Squitieri F, Telenius H, Adam S, Sajoo A, Starr E, Heidberg A, Wolff G, Hayden MR: Molecular analysis of new mutations for Huntington's disease: intermediate alleles and sex of origin effects. Nature Genet 1993, 5:174–179

75. Laird CD: Proposed mechanism of inheritance and expression of the human fragile-X syndrome of mental retardation. Genetics 1987, 117:587–599

76. Harper PS: Congenital myotonic dystrophy in Britain. Arch Dis Child 1975, 50:505–521

77. Ridley RM, Frith CD, Crow TJ, Conneally PM: Anticipation in Huntington's disease is inherited through the male line but may originate in the female. J Med Genet 1988, 25:589–595

78. Read AP: Huntington's disease: testing the test. Nature Genet 1993, 4:329–330

79. Reyniers E, Vits L, De Boulle K, Van Roy B, Van Velzen D, de Graaff E, Verkerk AJMH, Jorens HZJ, Darby JK, Oostra B, Willems PJ: The full mutation in the FMR-1 gene of male fragile X patients is absent in their sperm. Nature Genet 1993, 4:143–146

80. Wohrle D, Hennig I, Vogel W, Steinbach P: Mitotic stability of fragile X mutations in differentiated cells indicates early post-conceptional trinucleotide repeat expansion. Nature Genet 1993, 4:135–139

81. Bell MV, Hirst MC, Nakahori Y, MacKinnon RN, Roche A, Flint TJ, Jacobs PA, Tommerup N, Tranebjaerg L, Froster-Iskenius U, Kerr B, Turner G, Lindenbaum RH, Winter R, Pembrey M, Thibodeau S, Davies KE: Physical mapping across the fragile X: hypermethylation and clinical expression of the fragile X syndrome. Cell 1991, 64:861–866

82. Oberlé I, Rousseau F, Heitz D, Kretz D, Kretz C, Devys D, Hanauer A, Boué J, Bertheas MF, Mandel JL: Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. Science 1991, 252:1097–1102

83. Laird CD: Possible erasure of the imprint on a fragile X chromosome when transmitted by a male. Am J Med Genet 1991, 38:391–395

84. Hansen RS, Gartler SM, Scott CR, Chen S-H, Laird CD: Methylation analysis of CGG sites in the CpG island of the human FMR1 gene. Hum Mol Genet 1992, 1:571–578

85. van der Mey AGL, Maaswinkel-Mooy PD, Cornellisse CJ, Schmidt PH, van de Kamp JJP: Genomic imprinting in hereditary glomus tumors: evidence for a new genetic theory. Lancet 1989, 2:1291–1294

86. Heutink P, van der May AGL, Sandkuijl A, van Gils APG, Bardoel A, Breedveld GJ, van Vliet M, van Ommen G-JB, Cornelisse CJ, Oosta BA, Weber JL, Devilee P: A gene subject to genomic imprinting and responsible for hereditary paragangliomas maps to chromosome 11q23-qter. Hum Mol Genet 1992, 1:7–10

87. Knudson AG: Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 1971, 68: 820–823

88. Comings DE: A general theory of carcinogenesis. Proc Natl Acad Sci USA 1973, 70:3324–3328

89. Toguchida J, Ishizaki K, Sasaki MS, Nakamura Y, Ikenaga M, Kato M, Sugimot M, Kotoura Y, Yamamuro T: Preferential mutation of paternally derived RB gene as the initial event in sporadic osteosarcoma. Nature 1989, 338:156–158

90. Blanquet V, Turleau C, de Grouchy J, Creau-Goldberg N: Physical map around the retinoblastoma gene: possible genomic imprinting suggested by NruI digestion. Genomics 1991, 10:350–355

91. Greger V, Passarge E, Hopping W, Messmer E, Horsthemke B: Epigenetic changes may contribute to the formation and spontaneous regression of retinoblastoma. Hum Genet 1988, 83:155–158

92. Sakai T, Toguchida J, Ohtani N, Yandell DW, Rapaport JM, Dryja TP: Allele-specific hypermethylation of the retinoblastoma tumor-suppressor gene. Am J Hum Genet 1991, 48:880–888

93. Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM: Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science 1984, 224:1121–1124

94. Seeger RC, Brodeur GM, Sather H, Dalton A, Siegal SE, Wong KY, Hammond D: Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastoma. N Engl J Med 1985, 313:1111–1116

95. Cheng JM, Hiemstra JL, Schneider SS, Naumova A, Cheung N-KV, Cohn SL, Diller L, Sapienza C, Brodeur GM: Preferential amplification of the paternal allele of the N-myc gene in human neuroblastomas. Nature Genet 1993, 4:191–193

96. Caron H, van Sluis P, van Hoeve M, de Kraker J, Bras J, Slater R, Mannens M, Voute PS, Westerveld A, Versteeg R: Allelic loss of chromosome 1q36 in neuroblastoma is of preferential maternal origin and correlates with N-myc amplification. Nature Genet 1993, 4:187–190

97. Sotelo-Avila C, Gooch III WM: Neoplasms associated with the Beckwith-Wiedemann syndrome. Perspec Pediatr Pathol 1976, 3:255–272

98. Junien C: Beckwith-Wiedemann syndrome, tumourigenesis and imprinting. Curr Opin Genet Dev 1992, 2:431–438

99. Weksberg R, Teshima I, Williams BRG, Greenberg CR, Pueschel SM, Chernos JE, Fowlow SB, Hoyme E, Anderson IJ, Whiteman DAH, Fisher N, Squire J: Molecular characterization of cytogenetic alterations associated with the Beckwith-Weidemann syndrome (BWS) phenotype refines the localization and suggests the gene for BWS is imprinted. Hum Mol Genet 1993, 2:549–556

100. Lubinsky M, Hermann J, Kouseff AL, Opitz JM: Autosomal dominant sex-dependent transmission of the Wiedemann-Beckwith syndrome. Lancet 1974, i:932

101. Koufos A, Grundy P, Morgan K, Aleck KA, Hadro T, Lampkin BC, Kalbakji A, Cavanee WK: Familial Wiedemann-Beckwith syndrome and a second Wilms' tumor locus both map to 11p15.5. Am J Hum Genet 1989, 44:711–719

102. Ping AJ, Reeve AE, Law DJ, Young MR, Boehnke M, Feinberg AP: Genetic linkage of Beckwith-Wiedemann syndrome to 11p15. Am J Hum Genet 1989, 44:720–723

103. Brown KW, Williams JC, Maitland NJ, Mott MG: Genomic imprinting and the Beckwith-Wiedemann syndrome. Am J Hum Genet 1990, 46:1000–1001

104. Turleau C, de Grouchy J, Chavin-Colin CF, Martelli H, Voyer M, Charlas R: Trisomy 11p15 and Beckwith-Wiedemann syndrome: a report of two cases. Hum Genet 1984, 67:219–221

105. Henry I, Bonaiti-Pellié C, Chehensse V, Beldjord C, Schwartz C, Utermann G, Junien C: Uniparental disomy in a genetic cancer-predisposing syndrome. Nature 1991, 351:665–667

106. Grundy P, Telzerow P, Paterson MC, Haber D, Herman B, Li F, Garber J: Chromosome 11 uniparental isodisomy predisposing to embryonal neoplasms. Lancet 1991, 338:1079–1080

107. Brown KW, Gardner A, Williams JC, Mott MG, McDermott A, Maitland NJ: Paternal origin of 11p15 duplications in the Beckwith-Wiedemann syndrome. A new case and review of the literature. Cancer Genet Cytogenet 1992, 58:66–70

108. Ferguson-Smith AC, Cattanach BM, Barton SC, Beechey CV, Surani MA: Embryological and molecular investigations of parental imprinting on mouse chromosome 7. Nature 1991, 351:667–670

109. Seizinger BR, Klinger HP, Junien C, Nakamura Y, LeBeau M, Cavanee W, Emmanuel B, Ponder B, Naylor S, Mitelman F, Louis D, Menon A, Newsham I, Decker J, Kaelbling M, Henry I, v Deimling A: Report of the committee on chromosome and gene loss in human neoplasia. Cytogenet Cell Genet 1991, 58: 1080–1096

110. Schroeder WT, Chao L-Y, Dao DT, Strong LC, Pathak S, Riccardi VM, Lewis WK, Saunders GF: Nonrandom loss of maternal chromosome 11 alleles in Wilms' tumors. Am J Hum Genet 1987, 40:413–420

111. Williams JC, Brown KW, Mott MG, Maitland NJ: Maternal allele loss in Wilms' tumor. Lancet 1989, 1:283–284

112. Pal N, Wadey RB, Buckle B, Yeomans E, Pritchard J, Cowell JK: Preferential loss of maternal alleles in

sporadic Wilms' tumor. Oncogene 1990, 5:1665–1668

113. Scrable H, Cavenne W, Ghavimi F, Lovell M, Morgan K, Sapienza C: A model for embryonal rhabdomyosarcoma tumorigenesis that involves genome imprinting. Proc Natl Acad Sci USA 1989, 86:7480–7484

114. Weksberg R, Shen DR, Fei YL, Song QL, Squire J: Disruption of insulin-like growth factor 2 imprinting in Beckwith-Wiedemann syndrome. Nature Genet 1993, 5:143–150

115. Wilkins RJ: Genomic imprinting and carcinogenesis. Lancet 1988, 1:329–331

116. Feinberg A: Genomic imprinting and gene activation in cancer. Nature Genet 1993, 4:110–113

117. Reeve AE, Eccles MR, Wilkins RJ, Bell GI, Millow LJ: Expression of insulin-like growth factor-II transcripts in Wilms' tumour. Nature 1985, 317:258–260

118. Scott J, Cowell J, Robertson ME, Priestley LM, Wadey R, Hopkins B, Pritchard J, Bell GI, Rall LB, Graham CF, Knott TJ: Insulin-like growth factor-II gene expression in Wilms' tumour and embryonic tissues. Nature 1985, 317:260–262

119. Haselbacher GK, Irminger J-C, Zapf J, Ziegler WH, Humbel RE: Insulin-like growth factor II in human adrenal pheochromocytomas and Wilms tumors: Expression at the mRNA and protein level. Proc Natl Acad Sci USA 1987, 84:1104–1106

120. Baccarini P, Fiorentino M, D'Errico A, Mancini AM, Grigioni WF: Detection of antisense transcripts of the insulin-like growth factor-2 gene in Wilms' tumor. Am J Pathol 1993, 143:1535–1542

121. Reik W, Surani MA: Genomic imprinting and embryonal tumours. Nature 1989, 338:112–113

122. Pachnis V, Brannan CI, Tilghman SM: The structure and expression of a novel gene activated in early mouse embryogenesis. EMBO J 1988, 7:673–681

123. Wiles MV: Isolation of differentially expressed human cDNA clones: similarities between mouse and human embryonal stem cell differentiation. Development 1988, 104:403–413

124. Poirier F, Chan C-TJ, Timmons PM, Roberson EJ, Evans MJ, Rigby PWJ: The murine H19 gene is activated during embryonic stem cell differentiation in vitro and at the time of implantation in the developing embryo. Development 1992, 113:1105–1114

125. Rachmilewitz J, Gileadi O, Eldar-Geva T, Schneider T, De-Groot N, Hochberg A: Transcription of the H19 gene in differentiating cytotrophoblasts from human placenta. Mol Reprod Dev 1992, 32:196–202

126. Han DK, Liau G: Identification and characterization of developmentally regulated genes in vascular smooth muscle cells. Circ Res 1992, 71:711–719

127. Davis RL, Weintraub H, Lassar AB: Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell 1987, 51:987–1000

128. Brannan CI, Dees EC, Ingram RS, Tilghman SM: The product of the H19 gene may function as an RNA. Mol Cell Biol 1990, 10:28–36

129. Brunkow ME, Tilghman SM: Ectopic expression of the H19 gene in mice causes prenatal lethality. Genes Dev 1991, 5:1092–1101

130. Peebles PT, Trisch T, Papageorge AG: Isolation of four unusual pediatric solid tumor cell lines. Pediatr Res 1978, 12:485

131. Garvin AJ, Re GG, Tarnowski BI, Hazen-Martin DJ, Sens DA: The G401 cell line, utilized for studies of chromosomal changes in Wilms' tumor, is derived from a rhabdoid tumor of the kidney. Am J Pathol 1992, 142:375–380

132. Hao Y, Crenshaw T, Moulton T, Newcomb E, Tycko B: Tumour-suppressor activity of H19 RNA. Nature 1993, 365:764–767