Strife in the germ line

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ABSTRACT The formation of germ cells, their progress through meiosis, and the earliest stages of development are times when the genes in normal organisms are in balanced conflict. One conflict is expressed as meiotic drive, a system which characteristically associated with low fertility. It is argued that the association between carcinoma *in situ* (CIS) and low fertility can be explained by assuming that some meiotic drive system is operating in the testis. In meiotic drive systems, single haploid sets of chromosomes are frequently prevented from contributing to the next generation. The progression of carcinoma *in situ* to the near triploidy of germ cell tumors is taken as supportive evidence that meiotic drive systems are operating during tumor formation. Another conflict system is the opposing interests of the genes inherited from each parent. In general, genes inherited from the father promote growth and those from the mother limit the growth of the normal conceptus. In germ cell tumors, it is not known if the chromosomes retain a memory of their parent.

KEY WORDS: *genomic imprinting, meiotic drive, seminoma, teratocarcinoma*

Introduction

This note steals ideas from evolutionary genetics to illuminate the origin, growth, and differentiation of germ cell tumors: these theories make explicit predictions about the genetic interactions within the germ cells and their immediate progeny. It may seem paradoxical to celebrate the career of a superb practical scientist with a theoretical efflorescence, but there is always the next step in a subject which has been made fascinating by the work of others.

What can definitely be said about mammalian germ cell tumors has been said many times. Barry Pierce's contribution was to scythe many of the theories which had both bloomed and started to go to seed by 1911 (Ewing, 1911; summarized in Damjanov, 1991). Pierce's decisive experiments and observations on seminoma, teratoma, teratocarcinoma, yolk sac carcinoma, and choriocarcinoma were perspicacious: it is their equivalent normal tissues which are now considered to be the sites of intensive internecine strife in the genome. Here, this strife is taken to account for features of these tumors.

The idea that organisms struggle with each other to pass on their genes to the next generation had replaced notions about the harmony of the living world by the start of this century. Within each organism, new conflicts are now recognized: they flow from the interaction between individuals, but the characters are genes and cells rather than individuals. Instead of talk about harmonious body design and the beauty of physiological coordination and control, the new speak stresses «selfish» genes and the organismal battle of the sexes resolves into the conflicting interests of genes inherited from the mother and the father. It will become apparent that these confrontations might be intense in the common cell types of human germ cell tumors.

One reason for turning to evolutionary genetics is that our comprehension of these tumors remains limited using other means. First, there has been intensive study of the growth requirements of seminoma and the presumed embryonal carcinoma stem cell of teratocarcinoma, but there has been little progress (see Engstrom *et al.*, 1991; Mummery and Weima, 1991, and references therein). Nobody has yet managed to propagate in culture either human primordial germ cells or seminoma, their presumed transformed derivatives: until the conditions for culture have been defined, it will be difficult to discover whether it is the primordial germ cells or their environment, or both, which are changed during the development of this tumor. There is some hope in the recent reports that mouse primordial germ cells can stagger through several population doublings in culture (Godin *et al.*, 1991; Matsui *et al.*, 1991). Human embryonal carcinoma cell gardening has had some success. Some lines, which have been cultured for a long time, can now go through three of four population doublings in serum free medium with the addition of known growth factors (e.g. Engstrom *et al.*, 1985; Biddle *et al.*, 1988; Mummery and Weima, 1991). However, the action of at least one of these exogenous growth factors gave little information about the tumorigenic phenotype: it was found to promote cell survival rather than to drive the cell cycle (Biddle *et al.*, 1988). It now appears that these cells require factors which are the special products of extra-embryonic cells (see Pera *et al.*, 1990, 1991, and references therein). In this case, progress depends on the chemical characterization of these compounds, which might sustain embryonal carcinoma cell growth either by driving the cell cycle,

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or by promoting cell survival, or by reducing the rate of their differentiation.

Second, the systematic study of mutational changes in known oncogenes has only just begun (see Oosterhuis *et al.*, 1991, and references therein). Clearly, time will tell if such comprehensive surveys will be informative about the growth of these tumors: it is likely that they will be informative about tumor progression.

The third approach is family studies, to identify the genes involved in genetic susceptibility: in a small minority of cases there is a substantially elevated familial risk and it is probable that this results from genetic susceptibility (e.g. Forman *et al.*, 1992, and references therein). Note that the genetic mutations identified by familial studies are not necessarily the same as the mutations which are most common in spontaneous forms of the same tumor: the elegant studies on colon carcinoma make this very clear (Fearon and Vogelstein, 1990; Kinzler *et al.*, 1991).

In conclusion, molecular techniques have started to give more information about these tumors, but we can not be confident that they will be informative about the initiation of the majority of these tumors. The analysis of genetic battles in the germ line, which is presented here, might lead to an understanding of germ cell tumor initiation, but it is unlikely to make any contribution to the events of tumor progression: germ cell tumors, unlike their normal counterparts, make no contribution to the next generation but rather they prejudice their hosts' chance of leaving a genetic mark, with their early onset in males.

Meiotic drive, infertility, and germ cell tumors

Meiosis and germ cell tumors

This section attempts to account for the association of germ cell tumors with low or abnormal sperm production in men. There is a 0.7% lifetime risk of testicular cancer in the Danish population, and this risk is elevated in infertile men or men with reduced sperm counts (reviewed in Giwercman *et al.*, 1991).

Carcinoma in situ (CIS)

Testicular carcinoma *in situ* (CIS) is recognized by the presence of large cells which share several histochemical features with the primordial germ cells or gonocytes in the fetal gonads (see Giwercman *et al.*, 1991, for primary references).

The current opinion is that most germ cell tumors in the testis develop from CIS; the most compelling observation is that full blown germ cell tumors develop in 70% of patients within 7 years of CIS detection. The nuclei of CIS cells have double the DNA content of their normal neighbors (Muller and Skakkebaek, 1981), and Oosterhuis *et al.* (1989) have provided extensive karyotypic data to support their view that abnormal germ cells first become tetraploid, and then chromosome loss occurs leading to the slightly hyper-triploid seminomas and the slightly hypo-triploid non-seminomatous germ cell tumors (NSGCTs). The data does not suggest that there is an exact loss of one haploid set of chromosomes, for some chromosomes are under-represented and others are over-represented in these karyotypes with near triploid chromosome numbers.

Take the opinion that the formation of germ cell tumors is a multi-step process (e.g. Damjanov, 1991). If the first event in germ cell carcinogenesis is the formation of CIS, then the second event may be the activation of the mechanisms which normally occur in pre-meiotic germ cells or during meiosis. Certainly the peaks of testicular germ cell tumor incidence are related to the post-natal and post-pubertal increase in gonadotrophin and/or testosterone production (Skakkebaek *et al.*, 1987). The karyotypes of ovarian teratomas suggest that these tumors can originate from cells in the first meiotic division or later (Linder *et al.*, 1975; Parrington *et al.*, 1986 and references therein).

If the normal events of pre-meiotic germ cell divisions and meiosis are involved in the origin of germ cell tumors, then next consider the extent to which these events are the subjects of gene conflicts: genes and chromosomes are marked down for death. The reason is that «selfish» genes can establish an advantage in the next generation if they subvert the previously fair segregation of meiosis.

Meiotic drive

Meiotic drive is a process by which a gene becomes over-represented in the products of meiosis: the gene and its allele do not segregate in the normal Mendelian manner and the segregation ratio is distorted. Well known examples of such selfish genes (Dawkins, 1976) are Segregation Distorter in *Drosophila* and genes within the *t* region of the mouse. Meiotic drive has been well discussed in articles published in *American Naturalist* 137 (pp. 281-456, 1991), in Lyttle (1991) and in Hurst and Pomiankowski (1991). To quote from the last of these:

«In those systems studied in detail, drive results from the interaction between two genetic loci: the drive locus itself *(D)* and the site sensitive to its action *(I)*. Each locus has two alleles, where lower case denotes the wild-type allele: *D,d* and *I,i*. *D* chromosomes are capable of causing drive, whereas *d* are not. Three conditions must be met for production of the drive phenotype. First, the homologous chromosome must be a responder that is sensitive to the drive element *(i)*. Second, the drive element must not drive against itself. Self-tolerance is achieved if the drive element is linked to an insensitivity allele *(I)*. The key assumption here is that the insensitivity suppresses drive in *cis* but has no effect in *trans*. The existence of separate, albeit closely linked, drive and sensitivity loci is known for SD in *Drosophila* and for *t* in mice. In both cases the insensitivity allele acts only in *cis* (Frischauf, 1985; Sandler and Golic, 1985). Finally, the two loci must map to the same chromosome. If they do not, the gametic distortion caused will not preferentially affect the drive element itself.»

Meiotic driver genes must have arisen several times in the past, spread through the population, eliminated all chromosomes carrying the sensitivity locus *(i)*, and then disappeared from view: meiotic drivers can not be detected if they are very efficient and if there is no cost. We know about meiotic drivers when their spread is costly and incomplete. The costs identified by Hurst and Pomiankowski (1991) include the reduction of sperm as heterozygotes eliminate half their gametes, and the biochemical costs in producing the driver product and the costs of immunizing against its action (functions of the *I* locus). These costs must partially account for the frequent, but not inevitable, reduction in fertility when the meiotic driver becomes homozygous: this phenomenon is observed in both Segregation Distorter (Temin and Marthas, 1984) and with the *t* locus (Lyon, 1986): male mice which are homozygous for any one of the three driver loci in the *t* region are sterile if one of the other driver loci are heterozygous (Lyon, 1991). The cost of «immunization» is established by the observation that the insensitive *(i)* allele gradually disappears from *Drosophila* populations when they breed in the absence of Segregation Distorter (Wu *et al.*, 1989).

Infertility and meiotic drive

Are human meiotic drivers sufficient to account for the association between partial infertility and germ cell tumors? The presence of such drivers may only be a partial explanation of the human tumors because any disturbance of gametogenesis is likely to increase tumor incidence and reduce sperm production. For instance, there are mutations, such as Sl^{J} and *ter* which increase the incidence of spontaneous teratocarcinoma formation in male mice but which do not obviously give segregation distortion (Stevens, 1974; Noguchi and Stevens, 1982; Noguchi and Noguchi, 1985). The mutation *ter* leads to a deficiency in primordial germ cells and high tumor incidence in *ter/ter* homozygote males, and appears to prolong the divisions of the pre-meiotic germ cells in *ter/+* males: however, the mutation segregates as a single Mendelian recessive (Noguchi and Noguchi, 1985). The observation that humans with Klinefelters syndrome have an elevated risk of teratoma formation also suggests that the general disruption of meiosis need not depend on a meiotic drive system. Thus, meiotic drivers are likely to be only one of a set of genes which provoke germ cell tumor formation.

As a meiotic driver spreads through the population, then a reduction of fertility is expected in the heterozygotes (see above). At the least, we would expect abnormalities of sperm production even if these do not lead to a dramatic reduction of fertility.

It was not obvious why homozygosity at the meiotic drive locus *(D)* might also lead to infertility given tight linkage with the insensitivity locus *(I)* (discussed in Lyon, 1986, 1991; Lyttle, 1991). Recently, it has been argued that the meiotic drivers of the *t*- locus system exploit a normal product which is required for spermiogenesis (Lyon, 1992). The deletion of a driver produces a phenotype which is very similar to a driver allele. The interpretation is that an active driver *(D)* provides a reduced level of a substance which is required for normal spermiogenesis. The insensitive allele *(I)* is now regarded as an allele which can operate efficiently at low levels of this substance (Lyon, 1992). Such a system clearly links meiotic drive to low fertility because it becomes central to the driver's action that it should reduce the fertility of the wild type allele. In the case of the Segregation Distorter system, deletion of the driver locus creates a wild type phenotype, showing that the driver produces a novel product. In this case a simple explanation is that the insensitivity locus can not cope with two doses of the driver product which acts in *trans* (discussed in Lyttle, 1991).

We do not know of meiotic drivers in humans which are in equilibrium (stable polymorphisms): apparently there are no drivers which are as abundant in the population as Segregation Distorter in *Drosophila* (1-3%) and the *t*-haplotypes in mice (10-20%). The problem in discovering meiotic drivers is that there may be many causes of segregation distortion. For instance, segregation distortion certainly occurs in Alport's syndrome, which is an association of hereditary renal disease and deafness (Shaw and Glover, 1961), and there is slight evidence for abnormal segregation in a syndrome characterized by abnormalities of the hand and heart (Holt-Olam syndrome, Gall *et al.*, 1966). It is however difficult to exclude preferential late death of the conceptus for epigenetic reasons as an explanation of the distortion. In population studies, some evidence for segregation distortion has been obtained, but there is some doubt about these associations because mothers at risk may be more likely to come to the attention of the doctor (see Nath *et al.*, 1992).

This lack of clear evidence for meiotic drivers in the human population must be balanced against the difficulty of detecting such drivers if they have minor effects on segregation frequencies (low penetrance). Certainly the extent of segregation distortion can be modified by many other unlinked genes in the Segregation Distorter in *Drosophila* and the *t* system of the mouse. The conclusions are that meiotic drivers might exist in humans, we know little about their frequency, and that their actions could account for the observed association between low fertility and germ cell tumors.

Fratricide of alleles and chromosomes

This section accounts for the early loss of chromosomes during the development of germ cell tumors (Oosterhuis *et al.*, 1989, 1990; Castedo *et al.*, 1991).

It is not inevitable that elimination of the normal gene or chromosome should occur at meiosis: in one sex chromosome drive system in the wood lemming, a sex chromosome may be eliminated before meiosis in the mitotic divisions of the primordial germ cells, and a similar disjunction of a sex chromosome may also occur in the creeping vole (Ohno *et al.*, 1967; Fredga *et al.*, 1977). These observations suggest that events occurring near the time of meiosis may lead to chromosome instability.

To start, consider the methods by which drivers *(D)* eliminate the driven *(i)* alleles or chromosomes. There must be a time at which the driven allele *(i)* is marked and a time of execution by the driver product. So, while the effects of meiotic drivers are mainly seen in the products of meiosis, there is no necessary relation between the time of action of the driver on the driven allele *(i)* and the time at which the phenotype is obvious. The known meiotic drivers of eukaryotes do not conduct a biased gene conversion of the competing allele, but rather they eliminate both the driven allele and a whole haploid chromosome set in its immediate vicinity. In the «ultra-selfish» case of the small supernumerary chromosome which bears the paternal sex ratio gene, the activity of this chromosome destroys the whole haploid set of chromosomes derived from the male except itself: this mischievous behavior is observed after fertilization in the parasitoid wasp *Nasonia* (Werren, 1991).

Given that allelocide in a two locus meiotic drive system normally plays out as the destruction of all the chromosomes associated with the sensitive allele *(i)*, it is likely that the partial spatial isolation of haploid chromosome sets from each other in meiosis provides secluded dark alleys for taking out whole haploid chromosome sets. For instance, there is evidence that Segregation Distorter in *Drosophila* acts during metaphase I but the phenotype is not clear until late in spermiogenesis when a cell membrane fails to form around many of the sensitive sperm which are eventually destroyed. The time of action of the *t* driver is not known: spermiogenesis can appear normal and it may be that the sensitive sperm show premature triggering of the acrosome reaction (Brown *et al.*, 1989), and certainly their ability to fertilize is severely reduced.

In conclusion, this feature of meiotic drive, the elimination of whole chromosome sets, would provide a neat mechanism for reducing chromosome number at an early stage of germ cell tumor formation. The reduction from near tetraploidy to near triploidy is most consistent with a system in which the driver locus *(D)* and the insensitivity locus *(I)* are not yet tightly linked.

Mechanisms of chromosome elimination

Lyttle (1991) has reviewed the extent to which different segregation distorter systems may share similar mechanisms. It is the case that some of the driver and insensitivity loci are contained in

heterochromatin but his current conclusion is that the method of eliminating chromosome sets is very diverse. More molecular analysis is required before we know if such systems will provide molecular clues for detecting drivers and the driven in the human genome.

Genomic imprinting and the growth of germ cell tumors

This section accounts for the vigorous growth of germ cell tumors: in particular it emphasizes the growth of cells which are similar to those of the extraembryonic membranes of the normal conceptus.

Tissue specific genomic imprinting

Gene imprinting is a process by which the tissue-specific expression of each pair of alleles or each pair of homologous chromosomes may be governed by the sex of the parent which transmitted that region of the genome. For instance, the paternal X-chromosome is preferentially inactivated in the visceral endoderm, the parietal endoderm, and the trophectoderm of the mouse conceptus: similarly the paternal X-chromosome is preferentially inactivated in the placenta of humans (see Chapman, 1986, for primary references on preferential X-inactivation).

Early in the analysis of imprinted chromosome regions it was noticed that two copies of a paternal chromosome region tended to have reciprocal effects when compared with two maternal copies of the same chromosome region (reviewed in Cattanach and Beechey, 1990, and references therein). This observation has now been amplified in a variety of ways (reviewed in Surani *et al.*, 1990). First, diploid parthenogenetic conceptuses (all maternal chromosomes) have disproportionately small placentas, while diploid androgenetic conceptuses (all paternal chromosomes) have a disproportionately large placenta: chimeric combinations of either type with normal diploids show that the cells tend to colonize those tissues which are enlarged when they develop alone. The chimeras also allow much longer development of the uniparental cells, so that further details can be picked out; for instance, the androgenetic cells make a major contribution to the muscles of the chimera. From these observations and subsequent studies on humans, it is possible to generalize and state that genes inherited from the father (paternal genes) tend to increase the size of the conceptus, while genes inherited from the mother (maternal genes) tend to have the opposite effect as judged by birth weight (for example: Prader-Willi and Angelman syndrome, e.g. Mascari *et al.*, 1992; Smeets *et al.*, 1992 and references therein; one form of childhood diabetes, Julier *et al.*, 1991). It is also probable that some of these «parental» effects can influence the growth and development of the early human conceptus, for androgenetic conceptuses develop as hydatidiform mole, with extraembryonic tissues predominant, while the ovarian teratomata characteristically lack or have reduced amounts of these tissues (reviewed in Clarke, 1990).

These observations neatly fitted a theory developed by Haig and Westoby (1989) to account for the growth patterns of endosperm in flowering plants. In short, they noted that the paternal and maternal genes would transmit more offspring to the next generation if they pursued different aims. The conditions for this conflict are that the partners are not obliged to pair for life, and that their offspring should preferentially drain resources from one parent. In the case of plants, the resources are drained from the sex that bears the ovule, and in viviparous animals it is the mother which is weakened by the uterine growth of her offspring.

Insulin-like growth factor-II and its sink

The paradigm case of genomic imprinting and conceptus growth is the activity of the locus for insulin-like growth factor-II (IGF-II) and of its sink, the locus for the insulin-like growth factor-II/mannose-6-phosphate receptor (IGF-II/Man-6-P receptor or type II IGF receptor: reviewed in Haig and Graham, 1991; for general references on IGFs see Schofield, 1992). The expression of the paternal IGF-II locus is abundant and widespread in the embryonic and extraembryonic tissues of the early mouse and human conceptus, while the maternal locus is principally expressed in the exchange tissues around the brain and spinal cord of the mouse (De Chiara *et al.*, 1991). In contrast, it is only the maternal IGF-II/Man-6-P receptor which is expressed in the embryo during early development (Barlow *et al.*, 1991): the H19 gene shows a similar pattern of maternal expression, but its functions are unknown (Bartolomei *et al.*, 1991).

Once again there is clear evidence that the paternal alleles drive growth, while the maternal alleles restrict growth of the conceptus. When the paternal IGF-II gene is deleted, then the mouse conceptus is just over half size (De Chiara *et al.*, 1990, 1991), while the embryos are greatly enlarged when the paternal IGF-II locus is duplicated (with other genes on distal chromosome 7: Ferguson-Smith *et al.*, 1991). These effects on growth are probably mediated by the action of IGF-II on the type I IGF receptor, and we must presume that the IGF-II/Man-6-P sink gets blocked with excess IGF-II protein. In contrast, the IGF-II/Man-6-P receptor probably restricts the growth of the conceptus. When the IGF-II/Man-6-P is not transcribed, then the mice which are born are 16% heavier than their normal littermates (Forejt and Gregorova, 1992). This weight advantage is not permanent, which suggests that this action of the IGF-II/Man-6-P receptor is mainly on fetal growth. In normal circumstances, this receptor presumably reduces the growth of the conceptus by its action on IGF-II. The reciprocity of the embryonic actions and imprinting of IGF-II and the IGF-II/Man-6-P receptor give strong support to the Haig and Westoby theory.

Germ cell tumors and embryogenesis

Genomic imprinting phenomena are relevant to the development of germ cell tumors to the extent that these tumors mimic the events of early embryogenesis (reviewed in Stevens, 1974). It is likely that these tumors start to grow in female LT mice from oocytes which undergo some form of parthenogenetic activation: the observation that human ovarian teratomas may have chromosome sets which can be derived from secondary oocytes suggests that their origin may be similar (e.g. Linder *et al.*, 1975; Parrington *et al.*, 1986, and references therein). It is uncertain whether human testicular NSGCTs mimic embryogenesis in their origins. Their mouse counterparts appear to by-pass the stage of trophectoderm formation in their origins, and we are unclear whether we should regard a human embryonal carcinoma cell as a disguised micro-egg, an odd primordial germ cell, or a totipotential embryonic stem cell (see discussions in Oosterhuis *et al.*, 1991).

Assume that some form of genomic imprinting occurs during the origin and growth of some germ cell tumors. Then it is important to understand imprinting events because:

1) The imprinted loci regulate the growth of the early mouse conceptus, and must surely influence the multiplication of their abnormal counterparts in teratomas, teratocarcinomas, and other developmental tumors which are dominated by cell types similar to those of the early conceptus. In this case, information about imprinted loci in humans will reveal the normal regulators of embryonic growth, and should thus finger the regulatory circuits

which are disturbed in the abnormal growth of embryonal carcinoma, yolk sac carcinoma, and choriocarcinoma in these NSGCTs.

2) The growth of «all paternal» testicular NSGCTs should follow different imprinting constraints when compared with the «all maternal» ovarian tumors.

There are two immediate problems in applying hypotheses about imprinting to this analysis of human germ cell tumors. First, no identified protein-coding gene has been shown to be imprinted in humans. Second, we are only beginning to understand the extent to which «all paternal» and «all maternal» mouse conceptuses display the same pattern of genomic imprinting as those which are known in normal fertilized conceptuses. To start the discussion, let the imprinted loci in the mouse also be imprinted in the same parental sense in humans.

Human IGF-II locus

The human IGF-II locus is on the short arm of chromosome 11, and the argument is that this locus is imprinted in humans and that it is also involved in the growth of human germ cell tumors.

There is some evidence that the IGF-II locus is involved in the growth of the Wilms' kidney tumor for one potential trans-repressor of IGF-II expression is the WT1 gene, which is characteristically lost or mutated in these tumors (see Little *et al.*, 1991; Drummond *et al.*, 1992), and there are abundant IGF-II transcripts in Wilms' tumors (Scott *et al.*, 1985; Irminger *et al.*, 1987). There are two lines of evidence that the chromosome region around the human IGF-II locus is imprinted. First, the maternal short arm of chromosome 11 tends to be lost in Wilms' tumor, and the paternal equivalent tends to be duplicated. This observation suggests that some paternal loci in this region promote the growth of the tumor, and it is a short odds bet that it may be the IGF-II locus itself. Second, a molecular marker close by the IGF-II locus co-segregates with a tendency to develop childhood diabetes when this marker is inherited from the father but not the mother (Julier *et al.*, 1991). The implication is that this marker is near a region which is differentially expressed when it is inherited from the father or the mother. Thus, there are substantial reasons for working on the assumption that the human IGF-II locus is expressed more abundantly when it is inherited from the father.

IGF-II and tumor growth

If the testicular NSGCTs do retain an imprint of their paternal origin, then they should produce excess levels of IGF-II transcripts, with all their copies of the gene active. While testicular germ cell tumors can display modest but detectable levels of IGF-II transcripts, these are not nearly as abundant as these transcripts in Wilms' tumor (Scott *et al.*, 1985; Irminger *et al.*, 1987; Engstrom *et al.*, 1991), and it is clear that embryonal carcinoma cells still require exogenous IGF-II or IGF-I to go through population doublings in cell culture. There is therefore currently no direct evidence that the expression of IGF-II has anything to do with the *in vivo* growth of these tumors.

Genomic imprinting clues have also been confusing in the case of the IGF-II/Man-6-P receptor. In the mouse, this receptor is only expressed from the maternal allele in the early embryo and its expression in the extra-embryonic membranes of the conceptus is not yet described. If the same locus followed the same expression rules in the human, then testicular embryonal carcinoma cells should not express this receptor: such cells in culture do (Engstrom *et al.*, 1985; Biddle *et al.*, 1988).

The first paradigm of genomic imprinting in the mouse has thus failed to be informative about the growth of human germ cell tumors. We do not know if this is because mice are different to humans, because some of the imprinted loci do not show their parent of origin activity until their «other parent» alleles are present, because the pseudo-embryogenesis of NSGCTs is so confused that genomic imprinting is never initiated, because gene imprinting on the paternal line may require the completion of spermatogenesis, or because we do not yet know the critically imprinted loci. It is also possible that IGF-II expression prejudices the growth of these tumors because there is evidence that this is the case in fibrosarcomas (Schofield *et al.*, 1991).

In conclusion, it must remain a matter of nightmares whether the phenomenon of genomic imprinting is critical to the growth of these tumors.

Conclusions

The formation of germ cells, their progress through meiosis, and the earliest stages of development are times when the genes in normal organisms are in balanced conflict.

One conflict is expressed as meiotic drive, a system characteristically associated with low fertility. It is argued that the association between carcinoma *in situ* (CIS) and low fertility can be explained by assuming that some meiotic drive system is operating in these patients.

In meiotic drive systems, single haploid sets of chromosomes are frequently prevented from contributing to the next generation. The progression of carcinoma *in situ* to the near triploidy of germ cell tumors is taken as supportive evidence that meiotic drive systems are operating during tumor formation.

Another conflict system is the opposing interests of the genes inherited from each parent. In general, genes inherited from the father promote growth and those from the mother limit the growth of the normal conceptus. In germ cell tumors, it is not known if the chromosomes retain a memory of their parent.

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