Testicular germ-cell tumors in a broader perspective

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TESTICULAR GERM-CELL TUMOURS IN A BROADER PERSPECTIVE

J. Wolter Oosterhuis and Leendert H. J. Looijenga

Abstract | The germ-cell tumours are a fascinating group of neoplasms because of their unusual biology and the spectacular therapeutic results that have been obtained in these tumours. Traditionally, this group of neoplasms is presented in an organ-oriented approach. However, recent clinical and experimental data convincingly demonstrate that these neoplasms are one disease with separate entities that can manifest themselves in different anatomical sites. We propose five entities, in which the developmental potential is determined by the maturation stage and imprinting status of the originating germ cell. Recent progress begins to explain the apparent unpredictable development of germ-cell tumours and offers a basis for understanding their exquisite sensitivity to therapy.

RETROPERITONEAL
Located at the back of the abdomen, behind the lining known as the peritoneum that covers organs such as the stomach, liver, and parts of the large and small intestine. Organs such as the pancreas and kidneys are retroperitoneal.

MEDIASTINAL
Anatomical space of the thorax. Organs such as the thymus and heart are found within the mediastinum.

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Human germ-cell tumours (GCTs) are a heterogeneous group of neoplasms, which occur in the gonads, both the ovaries and the testes, and in different extragonadal sites along the midline of the body — the RETROPERITONEAL and MEDIASTINAL regions, and the midline of the brain. This anatomical distribution is possibly related to the migration route of primordial germ cells (PGCs) during embryogenesis[1]. The germ-cell origin of some GCTs is evident from the expression of specific proteins, such as the germ-line-specific protein VASA[2]. In others, it can be indirectly deduced from their pattern of genomic imprinting (see below and BOX 1). GCT characteristics can either be due to the process of tumorigenesis or just a reflection of normal embryonal development, which contributes to the complexity of these tumours. The clinical course of GCTs depends on factors such as sex and age of the patient, and histological type, anatomical site and spread of the tumour (TABLE 1).

Based on epidemiology, clinical presentation, phenotypic characterization, chromosomal constitution and genomic imprinting, the group of testicular GCTs comprises three of the five GCT entities: the teratomas and YOLK-SAC TUMOURS of newborn and infants; the seminomatous and non-seminomatous tumours of adolescents and young adults; and the spermatocytic seminomas of the elderly[3,4]. This distinction has recently been recognized by the 2004 World Health Organization classification system for testicular GCTs[5]. These three groups of GCTs will be discussed, with emphasis on the testicular type II GCTs, as these have been most extensively studied.

To allow a better understanding of the biology of GCTs, we will briefly review the origin of PGCs, embryonic stem cells (ESCs), embryonic germ cells (EGCs) and embryonal carcinoma cells (ECCs) (FIG. 1).

Origin of ESCs, ECCs, PGCs and EGCs

Specification between trophoectoderm and the inner cell mass is the first differentiation observed during embryogenesis, and results in a blastocyst. At this developmental stage, before implantation, the cells of the inner cell mass are pluripotent. They have been isolated and subcultured as ESCs and cell lines have been derived from them. In particular, ESCs express a gene known to be crucial for early embryonic development — octamer-binding transcription factor 3/4 (OCT3/4; also known as POU5F1) — and, like all somatic cells, have a biparental pattern of genomic imprinting (BOX 1). This is in line with their ability to contribute to all lineages in chimeric mice, including the germ-cell line[6] and marks these cells as omnipotent. In addition, they can form pluripotent tumours, known as teratomas when transplanted into sygeneic

Summary

- Germ-cell tumours (GCTs) of all anatomical sites can be classified into five groups, characterized by their chromosomal complement and developmental potential.
- The most significant recurrent chromosomal aberrations in type I yolk-sac tumours are loss of 1p, 4 and 6q, and gain of 1q, 12(p13) and 20q. In type II seminomas and non-seminomatous GCTs, the most significant recurrent chromosomal aberrations are gain of 7, 8, 12p, 21 and X, and loss of chromosomes 1p, 11, 13 and 18. Aberrations of 12p are the only recurrent structural abnormalities in type II GCTs. In type III spermatocytic seminomas, gain of chromosome 9 is most common.
- The originating cell is most probably a primitive germ cell of which the developmental potential differs according to its stage of maturation and pattern of genomic imprinting.
- Animal models are available for the different groups of GCTs, except for the type II seminomas and non-seminomatous GCTs.
- An activating *KIT* mutation in codon 816 is an early pathogenetic event in bilateral testicular seminomas and non-seminomatous GCTs.
- The transcription factor OCT3/4, a characteristic of primordial germ cells and pluripotent stem cells, is a new and robust diagnostic marker for type II seminomas and non-seminomatous GCTs, including their intratubular precursor.
- Treatment sensitivity and resistance of GCTs probably correlates with retention and loss of embryonic characteristics (in particular, DNA-repair deficiency), respectively.

mice. The pluripotent stem cells of these tumours — the ECCs — are the neoplastic counterparts of ESCs. However, in contrast to ESCs, ECCs do not contribute to the germ-cell lineage in chimeric mice. This is probably due to the accumulation of chromosomal/genetic aberrations following continuous growth of mouse ECCs[7].

PGCs are first recognized during embryonic development at day 6.5 in the mouse and week 5–6 in humans[8], when stained for alkaline phosphatase or OCT3/4, for example (see below). More recently, PGCs have been fluorescently tagged, which allows detailed investigation of their behaviour *in vivo*. PGCs originate from the proximal EPIBLAST and migrate, under control of the KIT stem-cell-factor signalling pathway, along the midline of the body through the hindgut to the GENITAL RIDGE. At the same time, their number greatly increases by proliferation[9]. During normal development, mouse PGCs located in extragonadal sites and the ovary enter meiosis, which is specifically inhibited in the testis[10]. Extragonadal PGCs will die by BAX-dependent apoptosis[11]. Once in the genital ridge, PGCs are referred to as gonocytes. Depending on the sex-chromosomal constitution and corresponding microenvironment in the gonadal ridge, these cells will differentiate into either oocytes or pre-spermatogonia (FIG. 1). Continuous cultures derived from PGCs/gonocytes, which can be established before the mouse developmental age of 12.5 days, are classified as EGC lines[12,13]. These cells are pluripotent and able to form teratomas (and ECCs), in contrast to the original PGC[6], but do not retain their germ-cell phenotype. This phenomenon of (*in vitro*) activation of PGCs to pluripotency is referred to as reprogramming. It has been demonstrated in the 129-strain mouse model (see below) that reprogramming of PGCs can also occur *in vivo*, resulting in the development of a GCT[14]. These have been used extensively as models for human GCTs.

Box 1 | Genomic imprinting

Genomic imprinting is the phenomenon that the paternal and maternal sets of chromosomes have different functionality in mammals, due to parental-specific epigenetic modification of the genome. This modification is established during germ-cell development (FIG. 1). Somewhere between the stage of a primordial germ cell (PGC) and a spermatozoa (male) and an oocyte (female), the originally biparental pattern of genomic imprinting, present in the zygote, has to be erased, and a uniparental pattern has to be established, either paternal or maternal[8,9]. It has been shown that the chromosomal constitution (that is, the presence of the Y chromosome), determines whether an embryonic germ cell will undergo paternal imprinting[182].

The functional difference between the paternal and maternal haploid set of chromosomes comes from so-called imprinted genes, which are expressed from the paternal or maternal allele only, sometimes in a tissue-specific manner. Although the exact mechanistic basis of monoallelic expression is not solved yet, it is clear that it is based on epigenetic modifications of the genome, including DNA methylation and histone acetylation[183].

From an evolutionary point of view, the existence of genomic imprinting has been related to a "…tug of war between the sexes"[184]. The paternal set of chromosomes promotes survival of the individual offspring, whereas the maternal set of chromosomes favours survival of the species. Teleologically, genomic imprinting prevents spontaneous activation of an oocyte (parthenogenesis) with the life-threatening development of trophoblastic tissues in the ovary. In fact, oocytes will only form somatic (non-invasive) tissue, known as a dermoid cyst. Genomic imprinting has great impact on normal and abnormal development, with consequences for the understanding of non-neoplastic and neoplastic conditions, the practice of assisted reproduction[185], and cloning of animal and human cells[186].

The status of genomic imprinting of germ-cell tumours has proven useful in identifying their cell of origin, and in explaining their developmental potential (see main text). The dermoid cyst of the ovary and the HYDATIDIFORM MOLE illustrate this point. The purely somatic differentiation of the dermoid cyst and the exclusive trophoblastic differentiation of the hydatidiform mole are most probably due to the complete maternal imprinting of the dermoid cyst, and the complete paternal imprinting of the hydatidiform mole. This assumption is based on results of nuclear transfer experiments performed by Solter and McGrath, and Surani and co-workers in the eighties[187,188], whereby experimental gynogenotes predominantly give rise to outgrowth of somatic tissues of the embryo proper. Conversely, androgenotes predominantly give rise to the development of a trophoblast.

YOLK-SAC TUMOUR
Subtype of the non-seminomatous germ-cell tumour that histologically resembles the yolk sac. It can appear as a pure tumour or as a part of a mixed non-seminomatous germ-cell tumour.

EPIBLAST
Columnar epithelium that lines the floor of the amniotic sac. This layer generates endoderm and mesoderm by migration of cells through the primitive streak. The remaining cells form ectoderm.

GENITAL RIDGES
A paired fold in the dorsal lining of the abdominal cavity of the embryo in which the gonads develop.

HYDATIDIFORM MOLE
Benign gestational neoplasm, grossly presenting as a bunch of grapes.

PARTHENOGENETIC OOCYTES
Oocytes that progress beyond meiosis I without being fertilized.

Type I GCTs

Type I teratomas and yolk-sac tumours occur in the ovaries and testes; the sacrococcygeal and retroperitoneal regions; the head and neck; the pineal and hypothalamic-hypophyseal region of the brain; and, very rarely, other sites like the vagina. The incidence is about 0.12 per 100,000, without a clear rise during recent decades[15]. Sacral teratomas are the most frequent, occurring in 1 of 40,000 predominantly female newborns, and the most common solid tumour in the newborn. Clinically, type I teratomas in sites other than the ovary are virtually always benign, but they can progress when incompletely surgically removed to yolk-sac tumours, which have the potential to metastasize and must be treated with chemotherapy[16].

Cell of origin, genomic imprinting and animal models. The partially erased pattern of genomic imprinting of these tumours[17,18] suggests a germ-cell origin (BOX 1), and indicates that the cell of origin is a more immature germ cell than the cell of origin of the type II and III GCTs (see below and FIG. 2). The extragonadal and gonadal tumours show a similar pattern of genomic imprinting, irrespective of histology. The tumours with a biparental pattern of genomic imprinting could have originated from an early (not yet erased) PGC, although origin from an ESC with biparental genomic imprinting cannot be excluded (FIG. 1).

Mouse teratoma models are in keeping with the above-mentioned contention. The testicular teratomas arising in the inbred 129-strain of mice and the ovarian teratomas of inbred LT/Sv mice (both derived from germ cells), and the embryo-derived teratomas (derived from ESCs), are most similar to human type I GCTs[19–23]. Of note, transplantation of gonadal ridges in 129-strain mice results in the development of teratomas only when the mice are less than 12.5 days gestational age[24]. This parallels the time window for generating EGCs. The late-appearing yolk-sac tumour of the embryo-derived teratoma model[25] is similar to the type I teratomas recurring as a yolk-sac tumour in infants.

The LT/Sv teratomas have been shown to originate from PARTHENOGENETIC OOCYTES that have arrested in meiosis I[26]. The default pathway of extragonadal (and ovarian) germ cells is entry into meiosis I[10]. The fact that mutations in *Steel* (stem-cell factor) and *Ter*, which are related to disturbances in PGC development, also increase the risk for teratocarcinoma[6] indicates that alterations in PGC development are important. Moreover, the recently described PGC-specific *Pten*-knockout mouse is relevant[27]. These animals develop teratomas with a high frequency, and the PGCs in these animals proliferate more extensively and show an increased capacity to generate EGCs. Another relevant recent model is the fragile histadine triad (*Fhit*)- knockout mouse that is characterized by the development of type I

Table 1 | **The five types of germ-cell tumour**

Type	Anatomical site	Phenotype	Age	Originating cell	Genomic imprinting	Genotype	Animal model
I	Testis/ovary/ sacral region/ retroperitoneum/ mediastinum/ neck/midline brain/other rare sites	(Immature) teratoma/ yolk-sac tumour	Neonates and children	Early PGC/ gonocyte	Biparental, partially erased	Diploid (teratoma). Aneuploid (yolk-sac tumour): gain of 1q, 12(p13) and 20q, and loss of 1p,4 and 6q	Mouse teratoma
II	Testis	Seminoma/ non-seminoma	>15 years (median age 35 and 25 years)	PGC/gonocyte	Erased	Aneuploid (+/– triploid): gain of X, 7, 8, 12p and 21; and loss of Y, 1p, 11, 13 and 18	Not available
	Ovary	Dysgerminoma/ non-seminoma	>4 years	PGC/gonocyte	Erased	Aneuploid	Not available
	Dysgenetic gonad	Dysgerminoma/ non-seminoma	Congenital	PGC/gonocyte	Erased	Diploid/tetraploid	Not available
	Anterior mediastinum (thymus)	Seminoma/ non-seminoma	Adolescents	PGC/gonocyte	Erased	Diploid/tri-tetraploid	Not available
	Midline brain (pineal gland/ hypothalamus)	Germinoma/ non-seminoma	Children (median age 13 years)	PGC/gonocyte	Erased	Diploid/tri-tetraploid	Not available
III	Testis	Spermatocytic seminoma	>50 years	Spermatogonium/ spermatocyte	Partially complete paternal	Aneuploid: gain of 9	Canine seminoma
IV	Ovary	Dermoid cyst	Children/adults	Oogonia/oocyte	Partially complete maternal	(Near) diploid, diploid/tetraploid, peritriploid (gain of X, 7, 12 and 15)	Mouse gynogenote
V	Placenta/uterus	Hydatidiform mole	Fertile period	Empty ovum/ spermatozoa	Completely paternal	Diploid (XX and XY)	Mouse androgenote

PGC, primordial germ cell.

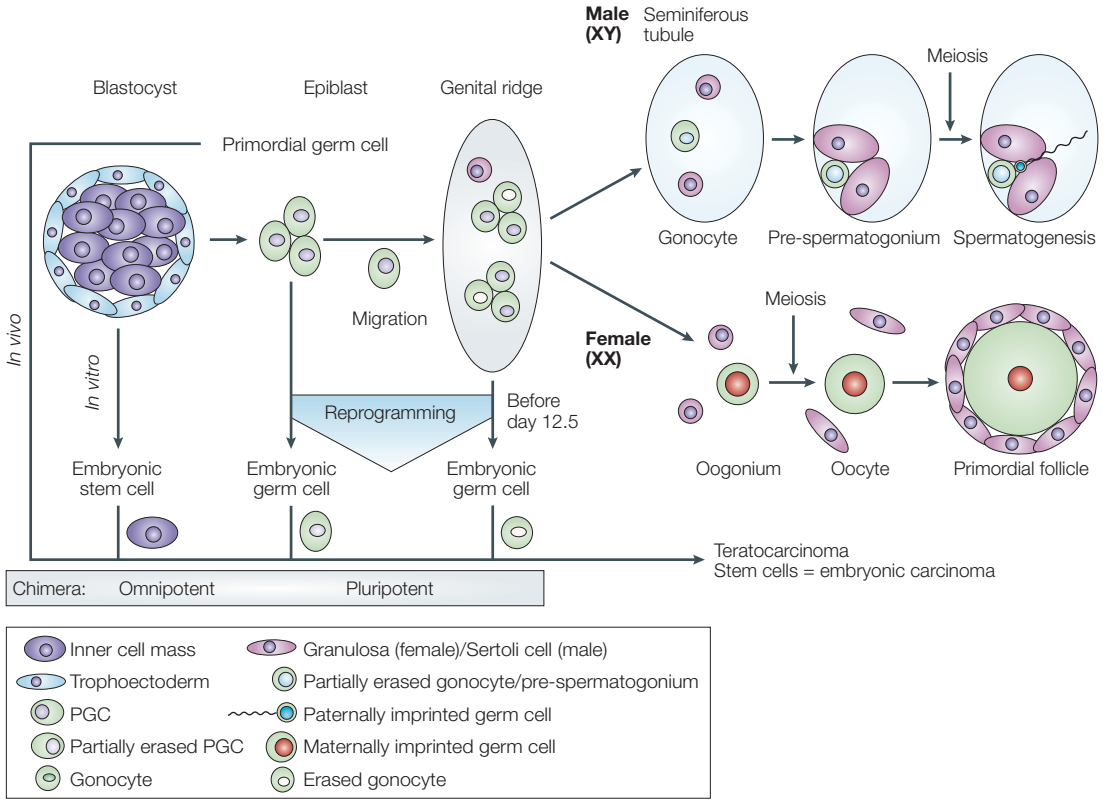

Figure 1 | **Schematic representation of normal embryonic development and origin of the germ-cell lineage.** The primordial germ cells (PGCs) originate in the epiblast, which can be identified by the alkaline phosphatase reactivity and by staining for octamer-binding transcription factor 3/4. These cells migrate to the genital ridge, after which they are referred to as gonocytes. They differentiate either to pre-spermatogonia or oocytes. Embryonic stem cells (ESCs) are derived from the inner cell mass, whereas embryonic germ cells (EGCs) can be isolated from PGCs until day 12.5 of development. The ESCs show a biparental pattern of genomic imprinting, whereas in EGCs this is erased. ESCs and EGCs can give rise to pluripotent teratomas, of which the embryonal carcinoma cells are the stem cells. Teratomas can also be formed directly from PGCs *in vivo*. During spermatogenesis, the paternal pattern of genomic imprinting is established, whereas the maternal pattern is formed during oogenesis. The timing of meiotic I arrest is different between male and female germ cells.

GCTs[28]. These mouse models indicate that disturbed control of maturation and apoptosis of PGCs both in extragonadal and gonadal sites might be involved in the pathogenesis of human type I GCTs.

Genomic constitution. Most human type I teratomas have a normal chromosomal complement[18,29]. By contrast, the type I yolk-sac tumours are aneuploid, with recurrent chromosomal changes, including loss of part of 1p, 4, 6q, and gains of parts of 1q, 12p (predominantly 12p13), 20q and 22 (REFS 18,29,30). These chromosomal changes occur in all anatomical localizations, supporting a common pathogenesis[31] (see below and TABLE 1). A peculiar variant of immature teratoma, presenting in adult males, is associated with tumour-specific complex translocations between chromosome 6 and chromosome 11; this teratoma is highly malignant and morphologically resembles type I teratoma[32,33].

In agreement with the above findings, high-resolution investigation of genomic imbalances in mouse ECC lines revealed specific chromosomal gains and losses. These include additional copies of chromosomes 6, 8, 12 and 15, and loss of parts of chromosomes 4, 6 and 14 (REF. 7). Gain of part of chromosome 6 is of particular interest because the human syntenic region is 12p13 — several genes involved in stem-cell biology map to this region, including *STELLAR* and *NANOG* (see below).

Type II GCTs

Histologically and clinically, type II GCTs are subdivided into seminomatous GCTs (called seminomas when occurring in the testis, dysgerminomas when occurring in the ovary or dysgenetic gonads, and germinomas when occurring in the brain) and non-seminomatous GCTs[34] (FIG. 3).

Epidemiology. Type II GCTs occur mainly in the gonads, particularly in the testis (referred to as TGCTs) and account for up to 60% of all malignancies diagnosed in men between 20–40 years of age[35]. In about 5% of these individuals, these tumours develop at extragonadal locations along the midline of the body, including the midline of the brain (pineal- and hypothalamic-hypophyseal region) and anterior mediastinum (thymus). The incidence of TGCTs

(between 6–11 per 100,000) has increased in Caucasian populations in recent decades[36], with an annual increase of 3–6%. For example, in Denmark and Switzerland the lifetime risk of developing a TGCT is up to 1%. Black populations have a significantly lower risk (which doesn't increase with age), with a similar histology and age distribution[37]. High oestrogen levels might increase the risk of developing a TGCT, whereas high testosterone levels reduce the risk[38], possibly explaining the different incidence rates in Caucasian and black populations[39].

The development of TGCTs is hypothesized to be part of the testicular dysgenesis syndrome, which includes CRYPTORCHIDISM and testicular atrophy, among other features[40,41]. This syndrome is thought to be the result of exposure to endogenous or environmental factors (in particular xeno-oestrogenic hormone disruptors) negatively affecting normal male gonadal development, particularly *in utero* (see below).

Pure seminomas comprise about 50% of TGCTs, with a median age of patients at diagnosis of 35 years. Non-seminomas develop earlier (median age at diagnosis of 25 years) and account for 30% of cases. The remaining are so-called combined tumours[42], which contain both seminoma and non-seminoma components and become clinically manifest at an intermediate age. Remarkably, cryptorchid testes predominantly develop seminomas[43], indicating that microenvironmental factors are involved in the regulation of the developmental potential of the precursor lesion. A predominance of dysgerminoma is observed in the ovarian type II GCTs.

Precursor lesion and cell of origin. All TGCTs originate from a precursor lesion, known as carcinoma *in situ*[44], also referred to as an intratubular germ-cell neoplasia unclassified (ITGCNU) lesion[34] — the term we shall use — and testicular intratubular neoplasia[45]. ITGCNU cells occupy the spermatogonial niche of the seminiferous tubule of the adult testis, in close connection with the basement membrane and Sertoli cells, the nursing cells of spermatogenesis (FIGS 1,3). This precursor is often present in the adjacent parenchyma of invasive TGCTs[46,47], and is more frequently associated with non-seminomas than seminomas. This is possibly due to the immunological response to seminoma cells, which also involves the ITGCNU cells, possibly because they are very similar to seminoma cells. This is apparent from lymphocytic infiltration of seminiferous tubules containing ITGCNU lesions, resulting in, sometimes complete, eradication of ITGCNU cells[47]. ITGCNU lesions are notably absent in testes with a type I GCT, testifying to the different pathogenesis of the type I and type II GCTs.

The incidence of ITGCNU lesions in the male Caucasian population is similar to the lifetime risk of developing a TGCT[48]. Apparently spontaneous regression of ITGCNU lesions does not occur, and eventually all patients with this lesion will develop an invasive TGCT. In about 50% of the patients this will happen within 5 years, and in 70% within 7 years[49].

Epidemiological observations[50] and the nature of the known risk factors for ITGCNU lesions — for example, familial predisposition, cryptorchidism, infertility, a previous TGCT and various forms of GONADAL DYSGENESIS — indicate that these lesions are the neoplastic counterpart of PGCs/gonocytes[51], initiated *in utero*. ITGCNU lesions are most likely due to delayed or blocked maturation of a PGC/gonocyte, possibly as part of the aforementioned testicular dysgenesis syndrome. In the testis[52] and ovary[53] this might be accompanied with tetraploidy in the affected germ cell. This origin of ITGCNU lesions is in line with the similarities in phenotype and ultrastructure between PGCs and ITGCNU[54] cells (FIG. 3). These cells also share similar patterns of genomic imprinting[55], telomerase activity[56] and gene expression[57]. The recently identified diagnostic marker for ITGCNU lesions (and some invasive TGCTs, see below) — nuclear expression of OCT3/4 — supports this model. After our first expression analysis[58] and subsequent immunohistochemical studies[59], the usefulness of this marker has been confirmed[60–64].

During normal embryonic development, mouse and human ESCs and PGCs show a nuclear staining for this transcription factor, which is involved in regulation of pluripotency[65]. OCT3/4 downregulation in both mouse and human ESCs results in a similar pattern of differentiation[66]. The finding that OCT3/4 expression in mouse ESCs is not influenced by ploidy and imprinting status[67] is in line with the observations in ITGCNU lesions and invasive type II GCTs (see below). Interestingly, a recent observation is that OCT3/4 has an apoptosis-suppressing effect in mouse PGCs, and no effect on the regulation of differentiation[68].

During normal intra-uterine and early post-natal maturation of germ cells OCT3/4 disappears, as do other markers of ITGCNU lesions, like placental/germ-cell alkaline phosphatase (PLAP) and KIT[63,69,70] (FIG. 3). Therefore, the results of staining of gonads in the first years of postnatal life must be interpreted with caution, because maturation-delayed (but non-malignant) germ cells might be positive for these markers. Misdiagnosis is therefore a risk in cryptorchidism and gonadal dysgenesis, conditions known for delay or arrest of germ-cell maturation. However, all ITGCNU cells are positive for OCT3/4, as are all seminomatous GCTs and embryonal carcinomas; following differentiation of embryonal carcinomas, OCT3/4 is downregulated[59,71]. OCT3/4 is also found in the germ-cell component of gonadoblastomas[72], the counterpart of ITGCNU lesions in the dysgenetic gonad[73]. *TSPY*, a gene possibly regulating proliferation, is a plausible candidate for the involvement of the Y chromosome in the development of this type of cancer[74].

At present, OCT3/4 is one of the most robust diagnostic markers for these cell types. It remains to be investigated whether epigenetic control is involved in the regulation of OCT3/4 in TGCTs, as found in ECCs[75] and mouse ESCs[76].

CRYPTORCHIDISM
The failure of the testes to descend into the scrotum.

GONADAL DYSGENESIS
Defective development of the gonads such as that seen in Turner's syndrome, where patients have only one complete X chromosome.

Phenotypic characterization. Seminoma/dysgerminoma/germinoma cells are morphologically similar to ITGCNU cells. Non-seminomas can contain different histological elements, including ECCs (the neoplastic counterpart of EGCs), teratoma cells (representing somatic differentiation), yolk-sac tumour cells, and choriocarcinoma cells (representing extra-embryonic differentiation). The default pathway of type II TGCTs is hypothesized to be towards the development of seminoma, and the development of a non-seminoma requires activation of pluripotency, also known as reprogramming of either an ITGCNU cell or a seminoma cell (FIGS 1,3). The more aggressive behaviour of non-seminomas explains why they become clinically manifest at a younger age than the more indolent seminomas. The phenomenon that seminomas appear in older patients is also referred to as 'loss of stem cell capacity'[77]. ITGCNU cells with stem-cell capacity probably become exhausted in older patient populations.

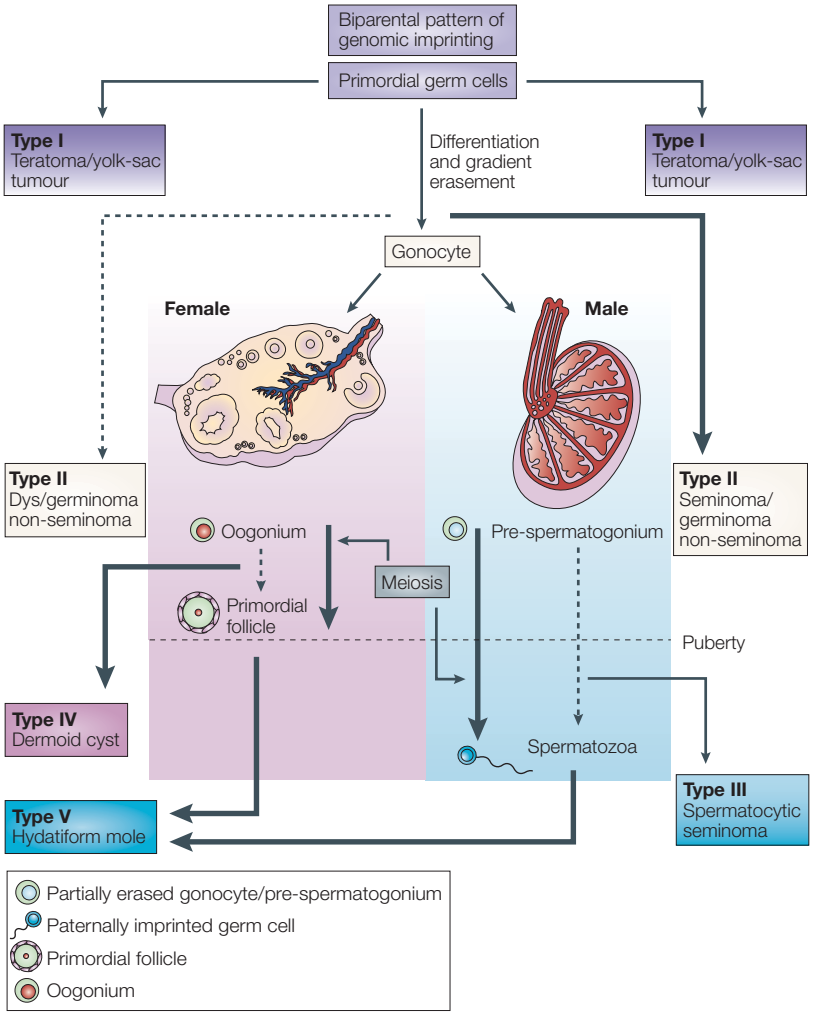

Figure 2 | **Schematic representation of the supposed origin of the various types of germ-cell tumour.** Normal germ-cell development, starting from a primordial germ cell (PGC), originally containing a biparental pattern of genomic imprinting, resulting in either an oocyte (female) or spermatozoa (male), is indicated in the middle panel. During this process, the biparental pattern of genomic imprinting is removed (erased), while a uniparental pattern is established, either female or male. The origin of the type I tumours is a PGC in extragonadal sites, or a gonocyte in the gonads, that underwent immediate reprogramming to become a pluripotent embryonic germ cell (EGC), the PGC-derived counterpart of an embryonic stem cell (ESC). It is highly likely that the cell of origin is in meiosis I arrest (Box 1). Type II germ-cell tumours originate only when the microenvironment favours survival and proliferation of (neoplastic) PGCs — that is, in the gonads, the thymus and in the pineal gland/hypothalamus/hypophyseal area. In these sites, the 'default' tumour is a seminoma/dysgerminoma/germinoma. The non-seminomas are the result of reprogramming of a seminomatous tumour cell to a pluripotent embryonal carcinoma cell (ECC), the malignant counterpart of the EGC/ESC. Note the different timing of initiation of meiosis between female and male development. The female germ cells enter meiosis during intra-uterine development (11–12 weeks of gestation), whereas for the male germ cells this only happens after the onset of puberty. This could explain the difference in incidence of the type II gonadal GCTs between females and males. In fact, the number of target cells (PGCs/gonocytes) for initiation is significantly lower in females compared with males. (Conversely, the target cells for type I GCTs are more frequent in the ovary than in the testis, where they do not exist beyond infancy. Corresponding with this, the ovarian type I GCTs are more frequent, and occur over much broader age range in females than in males.) The median age of clinical manifestation of type II GCTs in females is lower than in males. The type III tumour originates from a male germ cell, which underwent at least partial paternal imprinting. The type IV GCTs — dermoid cysts — originate most often from meiotic germ cells in the ovary that underwent maternal imprinting. The type V tumours originate from completely paternally imprinted mature male germ cells fertilizing an empty ovum.

A detailed morphological analysis of parenchyma adjacent to invasive TGCTs indicates that intratubular seminoma and intratubular non-seminoma are the intermediate stages between ITGCNU lesions and invasive growth[47] (FIG. 3). The intermediate stage of intratubular seminoma was independently confirmed[78]. The role of micro-invasive seminoma in the progression from an ITGCNU lesion to invasiveness is not yet clear, mainly due to the fact that it is poorly defined and remains to be investigated quantitatively[79]. Interestingly, intratubular non-seminoma is invariably composed of pure ECCs. Differentiation into its derivatives only starts after invasion (J.W.O., personal communication). An intriguing question is which factors trigger the differentiation when intratubular embryonal carcinoma becomes invasive?

ITGCNU cells, seminoma cells and ECCs show nuclear expression of OCT3/4 (see above). Staining for OCT3/4 in ITGCNU cells, seminomas and in the ECCs of non-seminomas is consistent with the observation that the type II GCTs are the only omnipotent cancers known (BOX 1; FIG. 3). It remains to be shown whether OCT3/4 expression is a driving force in the pathogenesis of this disease[60], or merely reflects the origin of the cancer and its developmental potential. A possible relationship between OCT3/4 expression and the active WNT–NOTCH pathway in ECCs remains to be elucidated[80].

The occurrence of EMBRYOID BODIES, resembling early human embryos in type II non-seminomas[34], together with specific patterns of gene expression and X-chromosome inactivation[81], illustrate the similarities between developmental patterns in the embryo and non-seminomas. Interestingly, the presence of unmethylated X-inactivation specific transcript (*XIST*) alleles in plasma, has been reported as a molecular marker for diagnosis and monitoring of patients with TGCTs[82].

Cytogenetics and epigenetics. TGCTs are consistently aneuploid, most likely because of the early establishment of polyploidy[52,83]. It is as yet unclear whether this aneuploidy is causally related to centrosome amplification[84]. One of the intriguing questions is whether exposure of PGCs/gonocytes to conditions of stress would induce polyploidy, as reported in yeast and flowering plants[85,86]. Polyploidy results in genomic instability, leading to genetic heterogeneity,

thereby inducing phenotypical changes within an initially phenotypically homogeneous population. This mechanism results in an enhanced evolution and selective survival of the best-adapted clone. However, in this context it is worth remembering that the seminomatous and non-seminomatous GCTs of the mediastinum[87] and dysgenetic gonads are mainly diploid[88,89], indicating that polyploidy is not essential for development of type II GCTs.

In the testis, the seminomas have a mean hypertriploid DNA index, whereas all histological components of non-seminoma have a mean hypotriploid DNA index[52], possibly due to net loss of chromosomal material during cancer progression[90]. This karyotype evolution seems to be rather specific for TGCTs[91] and has been reproduced using a computer model of multipolar cell division in a tetraploid cell[92]. A complex but highly consistent pattern of over- and under-representation of parts of chromosomes has been identified in seminomas and non-seminomas, both sporadic and familial, and confirmed in non-seminoma-derived cell lines[90,93–98]. Overall, relative loss of chromosomes 4, 5, 11, 13, 18 and Y, and gain of chromosomes 7, 8, 12 (in particular 12p, see below) and X is found. Differences between seminomas and non-seminomas relate to chromosomes 7, 15, 17, 19 and 22. These cytogenetic findings support a common origin of all histological subtypes of TGCTs. Moreover, mediastinal type II GCTs have a similar karyotype, indicating a similar pathogenesis[99].

Linkage analysis in families with a genetic predisposition to TGCTs indicates involvement of multiple genes with low penetrance[100]. The association between development of bilateral disease and cryptorchidism, related to a gene on the long arm of chromosome X, band q27, is highly interesting[101], although as yet unexplained.

The numerous studies on the possible role of tumour-suppressor genes in the origin of TGCTs will not be discussed here[4,102]. Although interesting observations have been made, none of the candidate genes have been definitively related to development of TGCTs.

All invasive type II GCTs show relative over-representation of 12p sequences. In up to 80% of the TGCTs this is due to one or more copies of ISOCHROMOSOME 12p (i(12p))[103,104]. GCTs without i(12p) show additional 12p sequences due to various structural anomalies[105,106], including high-level amplification (see below). Gain of 12p is also detected in similar tumours in extra-gonadal localities. This indicates that relative over-representation of 12p sequences is of crucial importance for the development of this cancer.

As well as genetic changes, epigenetic changes have been identified. In general, seminomas have demethylated DNA compared with non-seminomas[81,107–110]. It remains to be elucidated whether this merely reflects the omnipotency of TGCTs or is part of the changes leading to tumorigenesis.

Expression profiling of TGCTs. Not surprisingly, expression profiling showed distinct patterns for the different histological components of TGCTs[111]. Various known genes are overexpressed, including *CCND2* (encoding cyclin D2), as well as-yet-uncharacterized genes. Cyclin D2, predominantly expressed in the G1 phase of the cell cycle, is expressed in ITGCNU cells and most invasive TGCTs[112]. Cells lacking cyclin D are less prone to undergo malignant transformation. Interestingly, *CCND2* maps to the short arm of chromosome 12 band 13 (see below). The overexpressed genes growth factor receptor bound protein 7 (*GRB7*) and junction plakoglobin (*JUP*) are also of particular interest as they are localized on chromosome 17q. TGCTs[111] show a consistent gain of 17q sequences ,and the specific gain of chromosome 17 following continuous *in vitro* culture of human ESCs is also common (see below)[113]. GRB7 is a non-catalytic intracellular adaptor protein involved in cell migration. Overexpression of *GRB7* has been found in certain invasive and metastatic solid tumours. JUP is a cytoplasmic protein that is part of the submembranous plaques of DESMOSOMES and INTERMEDIATE JUNCTIONS. Recently, several of the proteins discussed above have been confirmed to be worthy of interest by a high-throughput approach[114].

High-throughput expression analyses have also demonstrated that ECCs are highly similar to ESCs, whereas seminoma cells are more similar to PGCs[61]. Such studies should help identify candidate genes for further analysis, and will further elucidate the cells of origin of this cancer[57].

It the context of the consistent gain of 12p sequences in these tumours, it is interesting that the expression profiles of human ESCs and ECCs mainly differ in that genes from chromosome 12 are overexpressed in ECCs[61].

Progression from ITGCNU lesions to invasiveness: role of chromosome 12. As well as the similar DNA ploidy of seminomas and ITGCNU lesions adjacent to an invasive TGCT[115], most of the chromosomal imbalances detected in the invasive tumour are also present in ITGCNU lesions. However, there are differences: ITGCNU lesions show loss of chromosomes 4 and 13, and gain of chromosome 2p[97,116–118]. Most important, no consistent gain of 12p is detected in ITGCNU lesions. These data indicate that over-representation of 12p is established during progression from pre-invasive to invasive behaviour[104] (FIG. 3). This is in keeping with the assumption that ITGCNU lesions are "…only one-step behind in the karyotypic evolution of TGCT"[119].

The number of 12p sequences in TGCTs does not correlate with stage of the disease and treatment resistance[120–122]. It is tempting to speculate that genes on 12p are involved in Sertoli-cell-independent/invasive growth of the tumour cells, consistent with the specific gain of chromosome 12 (and 12p) in ESC lines during continuous (semi-solid) *in vitro* culturing[113]. This underlines the growth advantage of pluripotent cells with over-representation of this genomic fragment. This model is also supported by the observation that gain of 12p is already present in intratubular seminomas and intratubular embryonal carcinomas, in which the tumour cells have lost their interaction with Sertoli cells but are still pre-invasive[121].

EMBRYOID BODIES
Cellular structures that arise when embryonic stem cells are cultured *in vitro*; they contain tissues from all three germ layers — endoderm, mesoderm and ectoderm.

ISOCHROMOSOME
An abnormal chromosome having a median centromere and two identical arms.

DESMOSOME
An adhesive junction that anchors intermediate filaments between adjoining cells.

INTERMEDIATE JUNCTIONS
Intermediate junctions are made of thickened cell membranes; the extra thickness is caused by polysaccharide, mostly hyaluronic acid, gluing the membranes together.

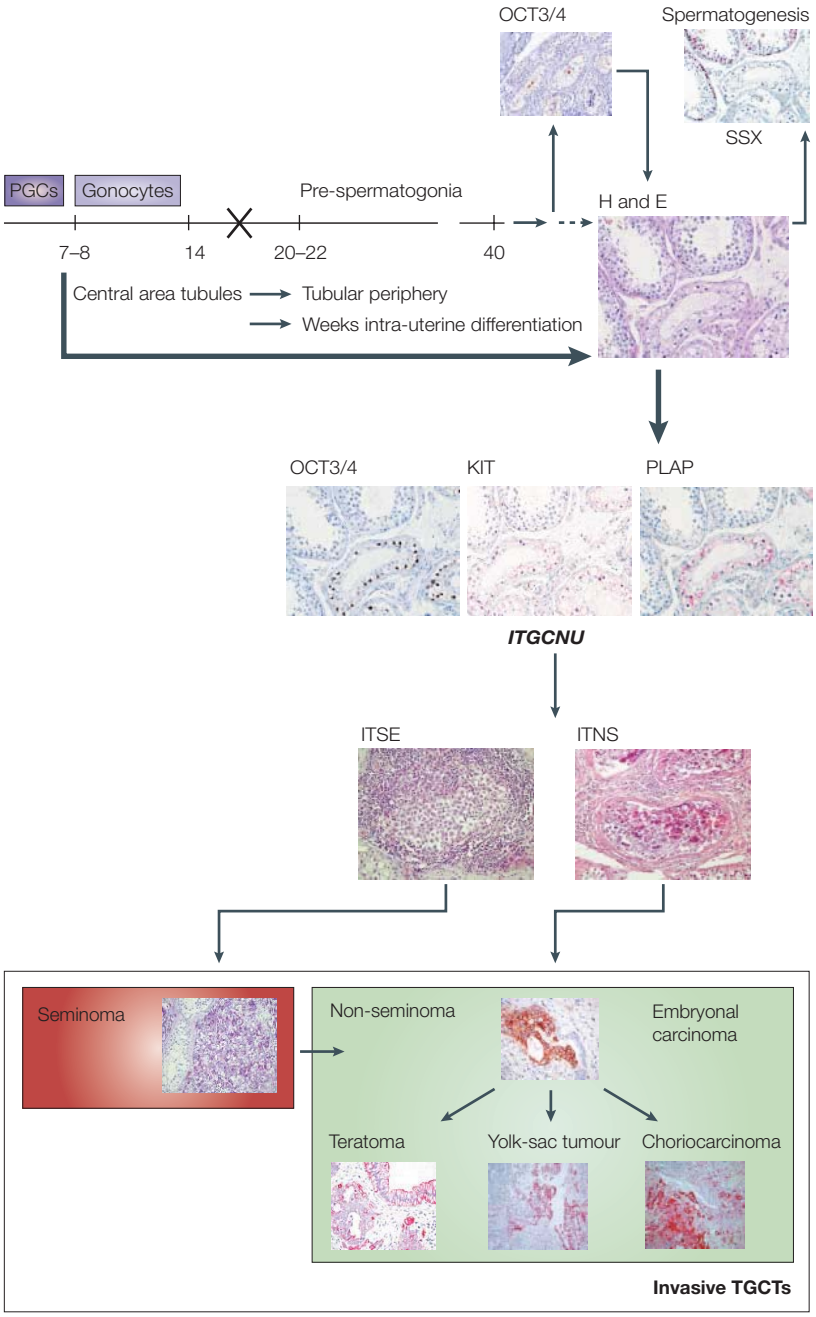

Figure 3 | **Characteristic differences between normal germ-cell development and formation of an ITGCNU lesion.** Normal intra-uterine development of male germ cells is indicated in the top left of the figure. Initially, the gonocytes are located in the lumen of the seminiferous tubules, and are positive for the different markers also found in intratubular germ-cell neoplasia unclassified (ITGCNU) lesions — like octamer-binding transcription factor 3/4 (OCT3/4). During the first year of life these cells can sporadically be found (top). After migration to the spermatogonial niche on the basal membrane (then referred to as pre-spermatogonia), they mature and loose their embryonic characteristics (including expression of OCT3/4), and will turn positive for markers of maturing spermatogenetic cells, like SSX (top right). ITGCNU lesions are composed of enlarged cells with clear cytoplasm; these cells have high mitotic activity and are located on a thickened basal membrane. These cells are characterized by the presence of glycogen (shown by periodic acid schift staining), alkaline phosphatase, placental/germ-cell alkaline phosphatase (PLAP), KIT and OCT3/4. The default pathway for ITGCNU cells is to gradually develop through intratubular seminoma to seminoma. A non-seminoma normally develops when an ITGCNU cell is reprogrammed to an (pluripotent) embryonal carcinoma cell — which is well characterized *in vitro*[179]. More rarely, a non-seminoma might develop through reprogramming of a seminoma cell[189]. This phenomenon explains the relatively frequent finding at autopsy of non-seminomatous metastases in patients originally diagnosed with a testicular seminoma[190]. ITNS, intratubular non-seminoma; ITSE, intratubular seminoma.

Various candidate genes on 12p have been investigated, including *KRAS*, *CCND2*, ethanolamine kinase 1 (*EKI1*), branched chain amino transferase 1 (*BCAT1*), lactate dehydrogenase B (*LDHB*) and, more recently, the stem-cell-specific genes *NANOG* and *STELLAR*[61,111,112,122–128]. The specific amplification in some tumours of a restricted region of 12p, including 12p11.2–12.1 and 12p13 might be instrumental to identify the relevant genes[122,129–131]. The 12p13 amplification is less well defined, although various candidates have been indicated, like *NANOG* and *STELLAR*[57,61,128]. High-level amplification of 12p11.2–p12.1 is predominantly found in seminomas, and exclusively in those lacking i(12p)[104,125]. This observation indicates that increased copy numbers of genes residing within that genomic fragment are beneficial for seminoma cells, most likely because of suppression of apoptosis[127]. The mutual exclusiveness of i(12p) and a restricted 12p amplification indicates the existence of two independent mechanisms resulting in gain of 12p sequences in (T)GCTs, one through i(12p) formation, and an alternative mechanism, which might result in restricted amplification. It is very interesting that a similar pattern involving 12p is found in pancreatic carcinomas[132], but it is not clear yet whether the same gene or genes are involved.

The current data support the model that multiple 12p genes are involved in the development of an invasive TGCT, although the exact nature of the mechanism remains unclear. Unfortunately, because of the lack of suitable model systems for ITGCNU lesions and seminomas, experimental testing of this hypothesis is not yet possible.

KIT and TGCTs. ITGCNU cells and seminoma cells, like PGCs[133], are characterized by staining for the stem-cell-factor receptor KIT[134] (FIG. 3). This tyrosine-kinase type III receptor is known to support survival of mouse PGCs[133,135] and seminoma cells *in vitro*[136]. TGCTs contain activating mutations in this gene in a low percentage of cases[137,138]. We showed that an activating codon 816 mutation, affecting the phosphotransferase region, is associated with the development of bilateral TGCTs[139]. So far, no link between presence of a *KIT* mutation and downstream target activation has been found. Our observation that the mutation detected in ITGCNU lesions can be absent in the matched invasive tumours indicates that the selective advantage of the mutation is significant in the *in situ* stage of the tumour, when the tumour cells are juxtaposed to Sertoli cells, which express membrane-bound KIT ligand. This explanation is consistent with our observation that the staining intensity for KIT is significantly less in seminomas than in ITGCNU lesions (J.W.O. and L.H.J.L., unpublished observations). The invasive seminoma cells have become independent from Sertoli cells, possibly because of the gain of genes on 12p that suppress apoptosis. The activating *KIT* mutation is the first genetic event demonstrated to have a role in the early, probably initiation, phase of type II GCTs. In view of its presence in bilateral tumours, the mutation must take place in PGCs before their arrival in the gonadal ridges.

The association between *KIT* codon 816 mutations and bilateral disease is clinically relevant, because about 2.5–5% of patients with TGCTs develop bilateral disease[140]. About half of the second tumours are diagnosed within the first 5 years after the initial diagnosis, although the latency period can be up to 20 years[141]. A second clinically manifest TGCT necessitates complete castration, leading to infertility, need for hormone substitution, and psychological burden. To prevent this situation, the contralateral testis is routinely biopsied in some countries, like Denmark and Germany, because detection of contralateral disease in the pre-invasive stage allows testis-sparing therapy[142]. By screening TGCTs for the KIT mutation, biopsy of the contralateral testis can be limited to the few percent of patients who have bilateral disease and who will eventually develop a second tumour. A prospective study on the predictive value of the mutation is in progress. Moreover, it might be a target for therapy with designed small molecules preserving in the one remaining testis not only hormonal function, but spermatogenesis as well.

Early diagnosis of TGCTs. Early diagnosis of ITGCNU lesions is feasible because of the long pre-invasive stage before clinical manifestation of the type II TGCTs. It is also clinically relevant because effective treatments for ITGCNU lesions are available. ITGCNU lesions can be eradicated with low-dose irradiation, which preserves the hormonal function in most patients[142], but results in infertility. By contrast, platinum-based chemotherapy has only a temporary effect on spermatogenesis[143]. Although 1% out of the general subfertile population has an ITGCNU lesion, we demonstrated that if bilateral MICROLITHIASIS is present (diagnosed using scrotal ultrasound) the risk for the presence of an ITGCNU lesion is up to 20%[144]. In agreement with this finding, patients with a TGCT and a contralateral ITGCNU lesion have a higher incidence of microlithiasis[145]. Bilateral microlithiasis and other risk factors like poor sperm count, small testis and age over 35 might help to select the best patients for biopsies[146]. The activating *KIT* mutation in codon 816 is not related to infertility. Screening for this mutation should be done in all patients with a TGCT.

Treatment sensitivity of type II GCTs. Seminomas are highly sensitive to both radiation and chemotherapy, whereas non-seminomas, by contrast, are apparently able to repair radiation-induced damage, typically double-strand breaks, and are therefore less susceptible to radiation. However, with the exception of teratomas, non-seminomas are highly sensitive to platinum-based combination chemotherapy. This might be explained by inherent properties of the cells from which they originate[147]. ESCs are particularly prone to cope with damaged DNA by elimination of irreparably damaged cells through apoptosis[148–150]. For germ cells, which share many properties with embryonic stem cells, this control mechanism can be expected to be similarly important to limit the risk that mutations are being passed on to the next generation. Loss of this embryonic feature might underlie the development of treatment resistance, which occurs in about 10–30% of patients diagnosed with a metastatic non-seminomatous type II GCT (see below).

It is now accepted that treatment sensitivity of type II GCTs is not determined by their p53 status[151–153]. The p53 levels probably reflect the origin from PGCs[154]. The overall drug sensitivity is likely due to factors downstream of DNA damage, possibly related to intrinsic or extrinsic pathways of apoptosis or to DNA-repair pathways. This might be related to a low DNA-repair capacity, associated with XPA (xeroderma pigmentosum, complementation group A)[155], although no direct evidence based on patient samples is available[156]. Other suggested mechanisms are related to testis-specific high mobility group box proteins[157], the FAS-ligand death receptor system[158], and levels of a serine/arginine-rich protein-specific kinase (SRPK1)[159]. In conclusion, the molecular basis for the exquisite and unique treatment sensitivity of type II GCTs is unexplained so far, but it is likely to be related to the intrinsic characteristics of the PGCs/gonocytes from which these tumours originate.

Treatment resistance of type II GCTs. Ten to thirty percent of patients diagnosed with metastatic non-seminomas will not achieve a durable complete remission after initial treatment, either because of incomplete response or relapse. This is most reliably predicted by the prognostic classification developed by the International Germ Cell Cancer Collaborative Group[160], in which the serum tumour markers α-fetoprotein, human chorionic gonadotropin and LDH, tumour stage and anatomical site of the primary tumour are key elements. Patients with an extragonadal seminoma have a long-term chance of cure similar to patients with a testicular primary tumour, but patients with a mediastinal non-seminoma show a significantly inferior outcome. In fact, they constitute the group with the worst prognosis of all — poor prognosis GCT patients — and outcome is even worse than in TGCT patients with multiple metastases[161].

Mature teratomas do not share the general chemosensitivity of GCTs to cisplatin-based combination chemotherapy. This component of type II GCTs has lost embryonic characteristics, as it is composed of terminally differentiated somatic tissues. This explains why so-called residual mature teratoma is found in about 30–40% of remnants of metastases after chemotherapy. In this context, it is of interest that mature teratomas express RB, p21 (also known as WAF1) and p27 (also known as KIP1), indicating that these lesions, in contrast to less differentiated elements, are able to go into G_1/S cycle arrest[162]. Interestingly, cells lacking p21 had a reduced ability to repair cisplatin-induced DNA damage and showed an increased sensitivity to this agent[163]. The presence of teratoma elements in the primary tumour predicts residual mature teratoma[164].

Resistance of type II GCTs might be related to a defect DNA mismatch-repair pathway — leading to microsatellite instability[165] — or a defective caspase-9 function[166]. The idea that resistance is related to epigenetic regulation of gene expression is supported by the finding that

MICROLITHIASIS
The formation of minute deposits of mainly inorganic material in tissues; for example, in the seminiferous tubules of the testis.

mature teratomas express a range of transporters from the ATP-binding cassette superfamily; for example, multidrug resistance (MDR), MDR-associated protein 2, breast cancer resistance protein, lung resistance protein and glutathione *S*-transferase[167]. In addition, unlike the other GCT-components, mature teratomas have a low BAX/BCL2 ratio, which indicates a decreased sensitivity to induction of apoptosis[167]. Further evidence that induction of differentiation has a role in treatment resistance of GCTs comes from a study of *in vitro* exposure of the ECC line Tera-2 to the differentiation inducer retinoic acid[168].

In conclusion, the chemoresistance of mature teratomas compared with the other histological elements of type II GCTs might be due to their intrinsic capacity to respond to DNA damage. As a result of their terminal somatic differentiation, they can respond to cisplatin-induced DNA damage by cell-cycle arrest rather than undergoing apoptosis. By contrast, the undifferentiated cells lack this capacity[54], as do fetal gonocytes and ITGCNU cells[169,170]. The mechanistic basis of the cisplatin resistance of non-teratomatous elements of type II GCTs remains to be elucidated.

Type III GCTs: the spermatocytic seminomas

The incidence of spermatocytic seminoma is about 0.20 per 100,000, without a clear rise during recent decades[171–173]. This exclusively testicular tumour, which is bilateral in 5% of cases, occurs predominantly in patients who are over 50 years of age. Spermatocytic seminomas are, for all practical purposes, benign tumours that can be cured by orchidectomy. As an exceptionally rare event they can progress to give rise to sarcomas. Reports on metastatic spermatocytic seminomas have to be considered with caution because of the pitfall of misdiagnosis as seminoma.

Phenotypic characterization, cell of origin and animal models. Spermatocytic seminoma is morphologically characterized by the presence of small, intermediate and large cells with similarities to spermatogonia and spermatocytes. Morphologically, spermatocytic seminomas might be misdiagnosed as seminomas, but this can now easily be avoided with the help of (immuno)histochemistry[174–176]. Positive staining for SSX, XPA, synaptonemal complex protein 1, CHK2, melanoma antigen family A4 and neuron-specific enolase, and a negative result for KIT, PLAP and OCT3/4, distinguishes spermatocytic seminoma from seminoma. Its morphology indicates that spermatocytic seminoma is derived from a more mature germ cell. This is in line with the observation that intratubular spermatocytic seminoma is the precursor lesion of this tumour, indicating that the cell undergoing the final step to neoplastic transformation is situated at the luminal side of the tight junctions connecting Sertoli cells. Some of the expressed markers and shared cytogenetic aberrations in bilateral tumours[177] are more compatible with a less mature cell of origin — probably a PGC before reaching the gonadal ridges. A PGC could acquire an initial mutation that does not affect the maturation of this cell to the stage of the spermatocyte. Further steps in the pathogenetic process occur once this cell has reached the luminal side of the tight junctions connecting the Sertoli cells.

Canine seminomas can be regarded as an animal counterpart of the type III GCTs[178]. It remains to be seen whether this is also true for the PUF-8 mutant induced GCTs in *Caenorhabditis elegans*[179]. PUF proteins are a conserved family of RNA-binding proteins that regulate RNA stability and translation by binding to specific sequences in 3′-untranslated regions. *Drosophila* PUMILIO and *C. elegans* FBF are representatives of this group of proteins, which are found to be essential for self-renewal of germline stem cells, indicating that a common function of PUF proteins might be to sustain mitotic proliferation of stem cells.

Cytogenetics and epigenetics. Data on the chromosomal constitution of spermatocytic seminoma are scarce. The published results indicate that structural chromosomal changes are rare, and that gain of chromosome 9 is the only recurrent imbalance[180]. The paternal pattern of genomic imprinting of spermatocytic seminoma is consistent with this tumour arising from a germ cell that is more mature than a PGC[181] (J.W.O. and L.H.J.L., unpublished observations).

Conclusions and future perspectives

Based on recent literature and our own data, we propose that the various types of GCT, however heterogeneous from many points of view, are in fact one disease, reflecting the developmental potential of germ cells in different stages of maturation, and with different imprinting status. In view of their similar developmental potential, the type I teratomas and yolk-sac tumours are most likely the human counterpart of the spontaneous and experimental teratomas of the testis of the 129-strain mice, the ovarian teratomas of the LT/Sv mouse, and the mouse embryo-derived teratomas. The origin of type I GCTs is most likely a PGC undergoing meiosis I arrest — at various stages of erasement — that has been reprogrammed to form a pluripotent EGC. They lack a germ-cell component (that is, a seminoma/dysgerminoma/germinoma), which probably requires complete erasement and a proper microenvironment for its development.

There is no animal model for the type II GCTs. These tumours only develop at sites that are able to prevent a PGC from entering meiotic arrest, and support survival of a PGC. Such conditions can be met in the gonads and, surprisingly, in some extragonadal sites — the thymus and the pineal gland, hypothalamus and hypophyseal regions of the brain. Their pattern of genomic imprinting is consistent with an erased cell of origin. By default, these precursor cells give rise to tumours composed of primitive germ cells — ITGCNU lesions, seminomas, dysgerminomas or germinomas. Activation to pluripotency of neoplastic primitive germ cells of these neoplasms gives rise to non-seminomatous GCTs in a process similar to the reprogramming of a PGC to an EGC *in vitro*. The factors causing reprogramming are as yet unknown.

The type III GCT is restricted to the germ-cell lineage, and originates from a partially paternally imprinted germ cell. We consider the type IV

GYNOGENOTES
Animals with maternal chromosomes only that are artificially created using nuclear transfer experiments. Experimental gynogenotes support outgrowth of the somatic tissues of the embryo proper.

ANDROGENOTES
Animals with paternal chromosomes only that are artificially created using nuclear transfer experiments. Androgenotes support the development of the trophoblast.

(dermoid cyst) and V (hydatidiform mole) GCTs as the human counterparts of the mouse GYNOGENOTES and ANDROGENOTES, generated by nuclear transfer experiments, explaining their unidirectional lineage of differentiation.

This model integrates recent insights into developmental biology, in particular germ-cell development, and the pathogenesis of GCTs, allowing us to ask more focused questions. For example, what are the precursor cells of the type II GCTs in the thymus and the pineal gland/hypothalamus? Markers to identify them in these organs are now available. It is hard to believe that these cells only survive there to give rise to GCTs. What could their function be? Which factors cause reprogramming of a seminomatous tumour cell to give rise to a non-seminomatous GCT? Why is intratubular non-seminoma always composed of ECCs, and which factors cause these cells to differentiate following invasion? Molecular research has already begun to provide new targets for diagnosis and, perhaps in the case of mutated KIT, molecular therapy. In residual teratomas, and possibly in GCTs in general, is resistance to chemotherapy due to loss of germ-cell/embryonal characteristics of the tumour cells, associated with loss of sensitivity to apoptosis and switching on of DNA-repair pathways? These are just a sample of the many questions that in principle can be answered with the new molecular and biochemical tools that are now available.

1. Wylie, C. Germ cells. *Cell* **96**, 165–174 (1999).
2. Zeeman, A. M. *et al.* VASA is a specific marker for both normal and malignant human germ cells. *Lab. Invest.* **82**, 159–166 (2002).
3. Oosterhuis, J. W., Looijenga, L. H. J., Van Echten-Arends, J. & De Jong, B. Chromosomal constitution and developmental potential of human germ cell tumors and teratomas. *Cancer Genet. Cytogenet.* **95**, 96–102 (1997).
First attempt to classify GCTs according to chromosomal constitution and developmental potential.
4. Looijenga, L. H. J. & Oosterhuis, J. W. Pathobiology of testicular germ cell tumors: views and news. *Analyt. Quant. Cytol. Histol.* **24**, 263–279 (2002).
5. Woodward, P. J. *et al.* in *World Health Organization Classification of Tumours. Pathology and Genetics of the Urinary System and Male Genital Organs.* (eds. Eble, J. N., Sauter, G., Epstein, J. I. & Sesterhann, I. A.) 217–278 (IARC, Lyon, 2004).
6. Donovan, P. J. & de Miguel, M. P. Turning germ cells into stem cells. *Curr. Opin. Genet. Dev.* **13**, 463–471 (2003).
7. Blelloch, R. H. *et al.* Nuclear cloning of embryonal carcinoma cells. *Proc. Natl Acad. Sci. USA* **101**, 13985–13990 (2004).
8. Surani, M. A. Reprogramming of genome function through epigenetic inheritance. *Nature* **414**, 122–128 (2001).
Critical review of the impact of genomic imprinting on the developmental program of ESCs and EGCs, and its significance for stem-cell transplantation.
9. McLaren, A. Primordial germ cells in the mouse. *Dev. Biol.* **262**, 1–15 (2003).
10. McLaren, A. & Southee, D. Entry of mouse embryonic germ cells into meiosis. *Dev. Biol.* **187**, 107–113 (1997).
***In vitro* co-culture experiments showing the dominant role of the microenvironment in making germ cells enter meiosis. The study demonstrates that a testis-specific factor is probably involved in preventing germ cells in the pre-pubertal testis to enter meiosis.**
11. Stallock, J., Molyneaux, K., Schaible, K., Knudson, C. M. & Wylie, C. The pro-apoptotic gene *Bax* is required for the death of ectopic primordial germ cells during their migration in the mouse embryo. *Development* **130**, 6589–6597 (2003).
This study, using fluorescently tagged mouse primordial germ cells *in vivo*, demonstrated that extragonadal localization leads to a BAX-dependent induction of apoptosis in these cells.
12. Matsui, Y., Zsebo, K. & Hogan, B. L. Derivation of pluripotential embryonic stem cells from murine primordial germ cells in culture. *Cell* **70**, 841–847 (1992).
First publication describing the *in vitro* conditions that are required to grow mouse primordial germ cells. *In vitro*, mouse PGCs undergo spontaneous reprogramming to pluripotent embryonal stem cells.
13. Shamblott, M. J. *et al.* Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc. Natl Acad. Sci. USA* **95**, 13726–13731 (1998).
First paper on *in vitro* growth of human primordial germ cells. The cells lose germ-cell characteristics, and undergo spontaneous reprogramming to pluripotent EGCs.
14. Stevens, L. C. Origin of testicular teratomas from primordial germ cells in mice. *J. Natl Cancer Inst.* **38**, 549–552 (1967).
First demonstration that mouse PGCs can give rise to teratomas.
15. Rescorla, F. J. Pediatric germ cell tumors. *Semin. Surg. Oncol.* **16**, 144–158 (1999).
16. Gobel, U. *et al.* Germ-cell tumors in childhood and adolescence. GPOH MAKEI and the MAHO study groups. *Ann. Oncol.* **11**, 263–271 (2000).
17. Bussey, K. J. *et al.* SNRPN methylation patterns in germ cell tumors as a reflection of primordial germ cell development. *Genes Chromosom. Cancer* **32**, 342–352 (2001).
18. Schneider, D. T. *et al.* Multipoint imprinting analysis indicates a common precursor cell for gonadal and nongonadal pediatric germ cell tumors. *Cancer Res.* **61**, 7268–7276 (2001).
19. Stevens, L. C. & Little, C. C. Spontaneous testicular teratomas in an inbred strain of mice. *Proc. Natl Acad. Sci. USA* **40**, 1080–1087 (1954).
20. Stevens, L. C. Experimental production of testicular teratomas in mice of strains 129, A/He, and their F1 hybrids. *J. Natl Cancer Inst.* **44**, 923–929 (1970).
21. Damjanov, I., Solter, D., Belicza, M. & Skreb, N. Teratomas obtained through extrauterine growth of seven–day old mouse embryos. *J. Natl Cancer Inst.* **46**, 471 (1971).
22. Solter, D., Dominis, M. & Damjanov, I. Embryo-derived teratocarcinomas: I. The role of strain and gender in the control of teratogenesis. *Int. J. Cancer* **24**, 770–772 (1979).
23. Walt, H., Oosterhuis, J. W. & Stevens, L. C. Experimental testicular germ cell tumorigenesis in mouse strains with and without spontaneous tumours differs from development of germ cell tumours of the adult human testis. *Int. J. Androl.* **16**, 267–271 (1993).
24. Stevens, L. C. Development of resistance to teratocarcinogenesis by primordial germ cells in mice. *J. Natl Cancer Inst.* **37**, 859–867 (1966).
25. Van Berlo, R. J. *et al.* Yolk-sac carcinoma develops spontaneously as a late occurrence in slow-growing teratoid tumors produced from transplanted 7-day mouse embryos. *Int. J. Cancer* **45**, 153–155 (1990).
26. Eppig, J. J., Wigglesworth, K., Varnum, D. S. & Nadeau, J. H. Genetic regulation of traits essential for spontaneous ovarian teratocarcinogenesis in strain LT/Sv mice: aberrant meiotic cell cycle, oocyte activation, and parthenogenetic development. *Cancer Res.* **56**, 5047–5054 (1996).
27. Kimura, T. *et al.* Conditional loss of PTEN leads to testicular teratoma and enhances embryonic germ cell production. *Development* **130**, 1691–1700 (2003).
28. Fujishita, T. *et al.* Development of spontaneous tumours and intestinal lesions in *Fhit* gene knockout mice. *Br. J. Cancer* **91**, 1571–1574 (2004).
29. Mostert, M. C. *et al.* Comparative genomic and *in situ* hybridization of germ cell tumors of the infantile testis. *Lab. Invest.* **80**, 1055–1064 (2000).
30. Perlman, E. J. *et al.* Genetic analysis of childhood endodermal sinus tumors by comparative genomic hybridization. *J. Pediatr. Hematol. Oncol.* **22**, 100–105 (2000).
31. Veltman, I., Schepens, M. T., Looijenga, L. H. J., Strong, L. C. & Geurts van Kessel, A. Germ cell tumours in neonates and infants: a distinct subgroup. *APMIS* **111**, 152–160 (2003).
32. Sinke, R. J. *et al.* Molecular characterization of a recurring complex chromosomal translocation in two human extragonadal germ cell tumors. *Cancer Genet. Cytogenet.* **73**, 11–16 (1994).
33. Van Echten-Arends, J. *et al.* Definition of a new entity of malignant extragonadal germ cell tumors. *Genes Chromosom. Cancer* **12**, 8–15 (1995).
34. Mostofi, F. K., Sesterhenn, I. A. & Davis, C. J. J. Immunopathology of germ cell tumors of the testis. *Semin. Diagn. Pathol.* **4**, 320–341 (1987).
35. Ulbright, T. M. Germ cell neoplasms of the testis. *Am. J. Surg. Pathol.* **17**, 1075–1091 (1993).
36. Swerdlow, A. J. in *Germ Cell Tumours IV* (eds. Jones, W. G., Appleyard, I., Harnden, P. & Joffe, J. K.) 3–8 (John Libbey, London, 1998).
37. Moul, J. W. *et al.* Testicular cancer in blacks. A multicenter experience. *Cancer* **73**, 388–393 (1994).
38. McGregor, J. A. *et al.* Salivary estriol as risk assessment for preterm labor: a prospective trial. *Am. J. Obstet. Gynecol.* **173**, 1337–1342 (1995).
39. Henderson, B. E., Bernstein, L., Ross, R. K., Depue, R. H. & Judd, H. L. The early *in utero* oestrogen and testosteron environment of blacks and whites: potential effects on male offspring. *Br. J. Cancer* **57**, 216–218 (1988).
40. Skakkebaek, N. E. Testicular dysgenesis syndrome. *Horm. Res.* **60** (Suppl. 3), 49 (2003).
41. Skakkebaek, N. E. Testicular dysgenesis syndrome: new epidemiological evidence. *Int. J. Androl.* **27**, 189–191 (2004).
42. Pugh, R. C. B. in *Pathology of the Testis* (ed. Pugh, R. C. B.) 245–258 (Blackwell, Oxford, 1976).
43. Ogunbiyi, J. O., Shittu, O. B., Aghadiuno, P. U. & Lawani, J. Seminoma arising in cryptorchid testes in Nigerian males. *East Afr. Med. J.* **73**, 129–132 (1996).
44. Skakkebæk, N. E. Possible carcinoma-*in-situ* of the testis. *Lancet* **II**, 516–517 (1972).
Paper that identified *carcinoma in situ* (ITGCNU) as the precursor of testicular GCTs of adolescents and adults.
45. Loy, V. & Dieckmann, K. P. Carcinoma *in situ* of the testis: intratubular germ cell neoplasia or testicular intraepithelial neoplasia? *Hum. Pathol.* **21**, 457–458 (1990).
46. Jacobsen, G. K., Henriksen, O. B. & Van der Maase, H. Carcinoma *in situ* of testicular tissue adjacent to malignant germ-cell tumors: a study of 105 cases. *Cancer* **47**, 2660–2662 (1981).
47. Oosterhuis, J. W. *et al.* Morphology of testicular parenchyma adjacent to germ cell tumours; an interim report. *APMIS* **111**, 32–42 (2003).
48. Giwercman, A., Müller, J. & Skakkebæk, N. E. Prevalence of carcinoma-*in situ* and other histopathological abnormalities in testes from 399 men who died suddenly and unexpectedly. *J. Urol.* **145**, 77–80 (1991).
49. Giwercman, A. & Skakkebæk, N. E. Carcinoma *in situ* of the testis: biology, screening and management. *Eur. Urol.* **23**, 19–21 (1993).
50. Møller, H. Decreased testicular cancer risk in men born in wartime. *J. Natl Cancer Inst.* **81**, 1668–1669 (1989).
The intra-uterine origin of type II GCTs was supported by the epidemiological observation that men conceived during the Second World War had a reduced risk for testicular GCTs.
51. Skakkebæk, N. E. *et al.* Germ cell cancer and disorders of spermatogenesis: an environmental connection? *APMIS* **106**, 3–12 (1998).
52. Oosterhuis, J. W. *et al.* Ploidy of primary germ cell tumors of the testis. Pathogenetic and clinical relevance. *Lab. Invest.* **60**, 14–20 (1989).
53. Oud, P. S. *et al.* DNA cytometry of pure dysgerminomas of the ovary. *Int. J. Gynecol. Pathol.* **7**, 258–267 (1988).
54. Rajpert-De Meyts, E. *et al.* The emerging phenotype of the testicular carcinoma *in situ* germ cell. *APMIS* **111**, 267–278 (2003).

55. Van Gurp, R. J. L. M., Oosterhuis, J. W., Kalscheuer, V., Mariman, E. C. M. & Looijenga, L. H. J. Human testicular germ cell tumors show biallelic expression of the *H19* and *IGF2* gene. *J. Natl Cancer Inst.* **86**, 1070–1075 (1994).
The first link between type II GCTs and the germ-cell lineage specific mechanism of genomic imprinting. The tumours showed biallelic expression of imprinted genes, and therefore originated from an erased germ cell.
56. Albanell, J. *et al.* Telomerase activity in germ cell cancers and mature teratomas. *J. Natl Cancer Inst.* **91**, 1321–1326 (1999).
57. Almstrup, K. *et al.* Embryonic stem cell-like features of testicular carcinoma *in situ* revealed by genome-wide gene expression profiling. *Cancer Res.* **64**, 4736–4743 (2004).
58. Palumbo, C. *et al.* Expression of the PDGF α-receptor 1.5 kb transcript, OCT-4 and c-KIT in human normal and malignant tissues. Implications for early diagnosis of testicular germ cell tumors and understanding regulatory mechanisms. *J. Pathol.* **196**, 467–477 (2002).
59. Looijenga, L. H. J. *et al.* POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. *Cancer Res.* **63**, 2244–2250 (2003).
60. Gidekel, S., Pizov, G., Bergman, Y. & Pikarsky, E. Oct-3/4 is a dose-dependent oncogenic fate determinant. *Cancer Cell* **4**, 361–370 (2003).
61. Sperger, J. M. *et al.* Gene expression patterns in human embryonic stem cells and human pluripotent germ cell tumors. *Proc. Natl Acad. Sci. USA* **100**, 13350–13355 (2003).
The first large expression-profiling study on human type II GCTs showing similarities between primordial germ cells and seminoma. Genes on chromosome 12p showed overexpression in embryonal carcinoma compared with embryonic stem cells.
62. Jones, T. D., Ulbright, T. M., Eble, J. N., Baldridge, L. A. & Cheng, L. OCT4 staining in testicular tumors: a sensitive and specific marker for seminoma and embryonal carcinoma. *Am. J. Surg. Pathol.* **28**, 935–940 (2004).
63. Rajpert-De Meyts, E. *et al.* Developmental expression of POU5F1 (OCT-3/4) in normal and dysgenetic human gonads. *Hum. Reprod.* **19**, 1338–1344 (2004).
64. Cheng, L. *et al.* OCT4: A novel biomarker for dysgerminoma of the ovary. *Am. J. Surg. Pathol.* **28**, 1341–1346 (2004).
65. Niwa, H., Miyazaki, J. & Smith, A. G. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nature Genet.* **24**, 372–376 (2000).
The crucial role of OCT3/4 in determining possible differentiation lineages is demonstrated in mouse embryonic stem cells. The level of expression is very strictly correlated with the differentiation status and lineage of the cell.
66. Hay, D. C., Sutherland, L., Clark, J. & Burdon, T. Oct-4 knockdown induces similar patterns of endoderm and trophoblast differentiation markers in human and mouse embryonic stem cells. *Stem Cells* **22**, 225–235 (2004).
67. Liu, L., Czerwiec, E. & Keefe, D. L. Effect of ploidy and parental genome composition on expression of Oct-4 protein in mouse embryos. *Gene Expr. Patterns* **4**, 433–441 (2004).
68. Kehler, J. *et al.* Oct4 is required for primordial germ cell survival. *EMBO Rep.* **5**, 1078–1083 (2004).
69. Honecker, F. *et al.* Pathobiological implications of the expression of markers of testicular carcinoma *in situ* by foetal germ cells. *J. Pathol.* **203**, 849–857 (2004).
70. Stoop, H. *et al.* Differentiation and development of human female germ cells during prenatal gonadogenesis: an immunohistochemical stdy. *Hum. Reprod.* (in the press).
71. Matin, M. M. *et al.* Specific knockdown of Oct4 and β2-microglobulin expression by RNA interference in human embryonic stem cells and embryonic carcinoma cells. *Stem Cells* **22**, 659–668 (2004).
72. Kersemaekers, A. M. *et al.* Identifies of germ cells at risk for neoplastic transformation in gonadoblastomas: an immunohistochemical study for OCT3/4 and TSPY. *Hum. Pathol.* (in the press).
73. Scully, R. E. Gonadoblastoma: a review of 74 cases. *Cancer* **25**, 1340–1356 (1970).
74. Lau, Y., Chou, P., Iezzoni, J., Alonzo, J. & Komuves, L. Expression of a candidate gene for the gonadoblastoma locus in gonadoblastoma and testicular seminoma. *Cytogenet. Cell Genet.* **91**, 160–164 (2000).
75. Deb-Rinker, P., Ly, D., Jezierski, A., Sikorska, M. & Walker, P. R. Sequential DNA methylation of the *Nanog* and *Oct-4* upstream regions in human NT2 cells during neuronal differentiation. *J. Biol. Chem.* 21 Dec 2004 (doi:10.1074/jbc.C400479200).
76. Hattori, N. *et al.* Epigenetic control of mouse *Oct-4* gene expression in embryonic stem cells and trophoblast stem cells. *J. Biol. Chem.* **279**, 17063–17069 (2004).
77. Skakkebaek, N. E. *et al.* Germ cell cancer and disorders of spermatogenesis: an environmental connection? *APMIS* **106**, 3–11 (1998).
78. Donner, J., Kliesch, S., Brehm, R. & Bergmann, M. From carcinoma *in situ* to testicular germ cell tumour. *APMIS* **112**, 79–88 (2004).
79. Von Eyben, F. E., Jacobsen, G. K., Rorth, M. & Von Der Maase, H. Microinvasive germ cell tumour (MGCT) adjacent to testicular germ cell tumours. *Histopathology* **44**, 547–554 (2004).
80. Walsh, J. & Andrews, P. W. Expression of Wnt and Notch pathway genes in a pluripotent human embryonal carcinoma cell line and embryonic stem cell. *APMIS* **111**, 197–210 (2003).
81. Looijenga, L. H. J., Gillis, A. J. M., Van Gurp, R. J. H. L. M., Verkerk, A. J. M. H. & Oosterhuis, J. W. X inactivation in human testicular tumors. XIST expression and androgen receptor methylation status. *Am. J. Pathol.* **151**, 581–590 (1997).
82. Kawakami, T., Okamoto, K., Ogawa, O. & Okada, Y. *XIST* unmethylated DNA fragments in male-derived plasma as a tumour marker for testicular cancer. *Lancet* **363**, 40–42 (2004).
83. Geurts van Kessel, A. *et al.* Chromosome 12q heterozygosity is retained in i(12p)-positive testicular germ cell tumor cells. *Cancer Genet. Cytogenet.* **40**, 129–134 (1989).
84. Mayer, F. *et al.* Aneuploidy of human testicular germ cell tumors is associated with amplification of centrosomes. *Oncogene* **22**, 3859–3866 (2003).
85. Galitski, T., Saldanha, A. J., Styles, C. A., Lander, E. S. & Fink, G. R. Ploidy regulation of gene expression. *Science* **285**, 251–254 (1999).
86. Comai, L. *et al.* Phenotypic instability and rapid gene silencing in newly formed *arabidopsis* allotetraploids. *Plant Cell* **12**, 1551–1568 (2000).
87. Oosterhuis, J. W. *et al.* Ploidy of malignant mediastinal germ-cell tumors. *Hum. Pathol.* **21**, 729–732 (1990).
88. Riopel, M. A., Spellerberg, A., Griffin, C. A. & Perlman, E. J. Genetic analysis of ovarian germ cell tumors by comparative genomic hybridization. *Cancer Res.* **58**, 3105–3110 (1998).
89. Kraggerud, S. M. *et al.* DNA copy number changes in malignant ovarian germ cell tumors. *Cancer Res.* **60**, 3025–3030 (2000).
90. Van Echten-Arends, J. *et al.* No recurrent structural abnormalities in germ cell tumors of the adult testis apart from i(12p). *Genes Chromosom. Cancer* **14**, 133–144 (1995).
91. Hoglund, M. *et al.* Multivariate analyses of genomic imbalances in solid tumors reveal distinct and converging pathways of karyotypic evolution. *Genes Chromosom. Cancer* **31**, 156–171 (2001).
92. Frigyesi, A. *et al.* A model for karyotypic evolution in testicular germ cell tumors. *Genes Chromosom. Cancer* **40**, 172–178 (2004).
93. Castedo, S. M. M. J. *et al.* Cytogenetic analysis of ten human seminomas (two of them lacking the i(12p)). *Cancer Res.* **49**, 439–443 (1989).
94. Rodriguez, E. *et al.* Cytogenetic analysis of 124 prospectively ascertained male germ cell tumors. *Cancer Res.* **52**, 2285–2291 (1993).
95. Ottesen, A. M. *et al.* Detection of chromosomal aberrations in seminomatous germ cell tumours using comparative genomic hybridization. *Genes Chromosom. Cancer* **20**, 412–418 (1997).
96. Summersgill, B. *et al.* Molecular cytogenetic analysis of adult testicular germ cell tumours and identification of regions of consensus copy number change. *Br. J. Cancer* **77**, 305–313 (1998).
97. Looijenga, L. H. J. *et al.* Comparative genomic hybridization of microdissected samples from different stages in the development of a seminoma and nonseminoma. *J. Pathol.* **19**, 187–192 (2000).
98. Kraggerud, S. M. *et al.* Genome profiles of familial/bilateral and sporadic testicular germ cell tumors. *Genes Chromosom. Cancer* **34**, 168–174 (2002).
99. Chaganti, R. S. K., Rodriguez, E. & Mathew, S. Origin of adult male mediastinal germ-cell tumours. *Lancet* **343**, 1130–1132 (1994).
100. Rapley, E. A., Crockford, G. P., Easton, D. F., Stratton, M. R. & Bishop, D. T. Localisation of susceptibility genes for familial testicular germ cell tumour. *APMIS* **111**, 128–133 (2003).
101. Rapley, E. A. *et al.* Localization to Xq27 of a susceptibility gene for testicular germ-cell tumours. *Nature Genet.* **24**, 197–200 (2000).
102. von Eyben, F. E. Chromosomes, genes, and development of testicular germ cell tumors. *Cancer Genet. Cytogenet.* **151**, 93–138 (2004).
103. Atkin, N. B. & Baker, M. C. Specific chromosome change, i(12p), in testicular tumours? *Lancet* **2**, 1349 (1982).
First demonstration, by conventional kayotyping, of i(12p) as specific chromosomal aberration in testicular GCTs. Subsequently, i(12p) was demonstrated by others in GCTs of similar histology in the ovary, the mediastinum and the midline of the brain (type II GCTs).
104. Looijenga, L. H. J. *et al.* Role of gain of 12p in germ cell tumour development. *APMIS* **111**, 161–173 (2003).
105. Suijkerbuijk, R. F. *et al.* Overrepresentation of chromosome 12p sequences and karyotypic evolution in i(12p)-negative testicular germ-cell tumors revealed by fluorescence *in situ* hybridization. *Cancer Genet. Cytogenet.* **70**, 85–93 (1993).
106. Rodriguez, E. *et al.* Molecular cytogenetic analysis of i(12p)-negative human male germ cell tumors. *Genes Chromosom. Cancer* **8**, 230–236 (1993).
107. Koul, S. *et al.* Characteristic promoter hypermethylation signatures in male germ cell tumors. *Mol. Cancer* **1**, 8 (2002).
108. Smith-Sorensen, B. *et al.* Frequent promoter hypermethylation of the O^6-methylguanine-DNA methyltransferase (*MGMT*) gene in testicular cancer. *Oncogene* **21**, 8878–8884 (2002).
109. Smiraglia, D. J. *et al.* Distinct epigenetic phenotypes in seminomatous and nonseminomatous testicular germ cell tumors. *Oncogene* **21**, 3909–3916 (2002).
110. Honorio, S. *et al.* Frequent epigenetic inactivation of the RASSF1A tumour suppressor gene in testicular tumours and distinct methylation profiles of seminoma and nonseminoma testicular germ cell tumours. *Oncogene* **22**, 461–466 (2003).
111. Skotheim, R. I. *et al.* New insights into testicular germ cell tumorigenesis from gene expression profiling. *Cancer Res.* **62**, 2359–2364 (2002).
112. Houldsworth, J., Reuter, V., Bosl, G. J. & Chaganti, R. S. K. Aberrant expression of cyclin D2 is an early event in human male germ cell tumorigenesis. *Cell Growth Differ.* **8**, 293–299 (1997).
113. Draper, J. S. *et al.* Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nature Biotechnol.* **22**, 53–54 (2004).
114. Skotheim, R. I. *et al.* Candidate genes for testicular cancer evaluated by *in situ* protein expression analyses on tissue microarrays. *Neoplasia* **5**, 397–404 (2003).
115. De Graaff, W. E. *et al.* Ploidy of testicular carcinoma *in situ*. *Lab. Invest.* **66**, 166–168 (1992).
116. Rosenberg, C., Van Gurp, R. J., Geelen, E., Oosterhuis, J. W. & Looijenga, L. H. Overrepresentation of the short arm of chromosome 12 is related to invasive growth of human testicular seminomas and nonseminomas. *Oncogene* **19**, 5858–5862 (2000).
This study shows that invasive type II GCTs consistently show over-representation of the short arm of chromosome 12 and that their common precursor lesion, ITGCNU, lacks it. Over-representation of 12p is apparently related to progression of the cancer.
117. Summersgill, B., Osin, P., Lu, Y. J., Huddart, R. & Shipley, J. Chromosomal imbalances associated with carcinoma *in situ* and associated testicular germ cell tumours of adolescents and adults. *Br. J. Cancer* **85**, 213–220 (2001).
118. Ottesen, A. M. *et al.* High-resolution comparative genomic hybridization detects extra chromosome arm 12p material in most cases of carcinoma *in situ* adjacent to overt germ cell tumors, but not before the invasive tumor development. *Genes Chromosom. Cancer* **38**, 117–125 (2003).
119. Oosterhuis, J. W. & Looijenga, L. H. J. The biology of human germ cell tumours: retrospective speculations and new prospectives. *Eur. Urol.* **23**, 245–250 (1993).
120. Rao, P. H. *et al.* Chromosomal amplification is associated with cisplatinum resistance of human male germ cell tumors. *Cancer Res.* **58**, 4260–4263 (1998).
121. Roelofs, H. *et al.* Restricted 12p-amplification and RAS mutation in human germ cell tumors of the adult testis. *Am. J. Pathol.* **157**, 1155–1166 (2000).
122. Zafarana, G. *et al.* Coamplification of DAD-R, SOX5, and EKI1 in human testicular seminomas, with specific overexpression of DAD-R, correlates with reduced levels of apoptosis and earlier clinical manifestation. *Cancer Res.* **62**, 1822–1831 (2002).
123. Olie, R. A. *et al.* N- and KRAS mutations in human testicular germ cell tumors: incidence and possible biological implications. *Genes Chromosom. Cancer* **12**, 110–116 (1995).
124. Bartkova, J., Rajpert-De Meyts, E., Skakkebaek, N. E. & Bartek, J. D-type cyclins in adult human testis and testicular cancer: relation to cel type, proliferaton, differentiation, and malignancy. *J. Pathol.* **187**, 573–581 (1999).
125. Rodriguez, S. *et al.* Expression profile of genes from 12p in testicular germ cell tumors of adolescents and adults associated with i(12p) and amplification at 12p11. 2-p12. 1. *Oncogene* **22**, 1880–1891 (2003).

126. Bourdon, V. *et al.* Genomic and expression analysis of the 12p11-p12 amplicon using EST arrays identifies two novel amplified and overexpressed genes. *Cancer Res.* **62**, 6218–6223 (2002).
127. Zafarana, G. *et al.* 12p-amplicon structure analysis in testicular germ cell tumors of adolescents and adults by array-CGH. *Oncogene* **22**, 7695–7701 (2003).
128. Clark, A. T. *et al.* Human *STELLAR*, *NANOG*, and *GDF3* genes are expressed in pluripotent cells and map to chromosome 12p13, a hotspot for teratocarcinoma. *Stem Cells* **22**, 169–179 (2004).
129. Mostert, M. C. *et al.* Identification of the crucial region of 12p overrepresentation in testicular germ cell tumors of adolescents and adults. *Oncogene* **16**, 2617–2627 (1998).
130. Henegariu, O. *et al.* Characterization of gains, losses, and regional amplification in testicular germ cell tumor cell lines by comparative genomic hybridization. *Cancer Genet. Cytogenet.* **148**, 14–20 (2004).
131. McIntyre, A. *et al.* Defining minimum genomic regions of imbalance involved in testicular germ cell tumors of adolescents and adults through genome wide microarray analysis of cDNA clones. *Oncogene* **23**, 9142–9147 (2004).
132. Heidenblad, M. *et al.* Detailed genomic mapping and expression analyses of 12p amplifications in pancreatic carcinomas reveal a 3. 5-Mb target region for amplification. *Genes Chromosom. Cancer* **34**, 211–223 (2002).
133. De Miguel, M. P., Cheng, L., Holland, E. C., Federspiel, M. J. & Donovan, P. J. Dissection of the c-Kit signaling pathway in mouse primordial germ cells by retroviral-mediated gene transfer. *Proc. Natl Acad. Sci. USA* **99**, 10458–10463 (2002).
Using a novel technical approach, the signalling pathways in mouse germ cells could be investigated in detail. The AKT kinase was found to be important for growth *in vitro*.
134. Rajpert-De Meyts, E. & Skakkebæk, N. E. Expression of the c-kit protein product in carcinoma-*in-situ* and invasive testicular germ cell tumours. *Int. J. Androl.* **17**, 85–92 (1994).
135. Donovan, P. J. Growth factor regulation of mouse primordial germ cell development. *Curr. Top. Dev. Biol.* **29**, 189–225 (1994).
136. Olie, R. A. *et al.* in *Advances in Biosciences Vol 91 Germ Cell Tumors III* (eds. Jones, W. G., Harnden, P. & Appleyard, I.) 95–106 (Pergamon, 1994).
137. Tian, Q., Frierson, H. F. Jr., Krystal, G. W. & Moskaluk, C. A. Activating c-*kit* gene mutations in human germ cell tumors. *Am. J. Pathol.* **154**, 1643–1647 (1999).
138. Kemmer, K. *et al.* KIT mutations are common in testicular seminomas. *Am J. Pathol.* **164**, 305–313 (2004).
139. Looijenga, L. H. J. *et al.* Stem cell factor receptor (c-KIT) codon 816 mutations predict development of bilateral testicular germ cell tumors. *Cancer Res.* **63**, 7674–7678 (2003).
140. Dieckmann, K. P. & Loy, V. Prevalence of contralateral testicular intraepithelial neoplasia in patients with testicular germ cell neoplasms. *J. Clin. Oncol.* **14**, 3126–3132 (1996).
141. Lajos, G., Gomez, F., Mihaly, B. & Istvan, B. The incidence, prognosis, clinical and histological characteristics, treatment and outcome of patients with bilateral germ cell testicular cancer in Hungary. *J. Cancer Res. Clin. Oncol.* **129**, 309–315 (2003).
142. Rorth, M. *et al.* Carcinoma *in situ* of the testis. *Scan. J. Urol.* **205**, 166–186 (2000).
143. Gaffan, J. *et al.* Infertility rates following POMB/ACE chemotherapy for male and female germ cell tumours — a retrospective long-term follow-up study. *Br. J. Cancer* **89**, 1849–1854 (2003).
144. De Gouveia Brazao, C. A. *et al.* Bilateral testicular microlithiasis predicts development of malignant testicular germ cell tumours in subfertile men. *J. Urol.* **171**, 158–160 (2004).
145. Holm, M., Hoei-Hansen, C. E., Rajpert-De Meyts, E. & Skakkebaek, N. E. Increased risk of carcinoma *in situ* in patients with testicular germ cell cancer with ultrasonic microlithiasis in the contralateral testicle. *J. Urol.* **170**, 1163–1167 (2003).
146. Heidenreich, A. & Moul, J. W. Contralateral testicular biopsy procedure in patients with unilateral testis cancer: is it indicated? *Semin. Urol. Oncol.* **20**, 234–238 (2002).
147. Oosterhuis, J. W., Andrews, P. W., Knowles, B. B. & Damjanov, I. Effects of *cis*-platinum on embryonal carcinoma cell lines *in vitro*. *Int. J. Cancer* **34**, 133–139 (1984).
148. Cairns, J. Somatic stem cells and the kinetics of mutagenesis and carcinogenesis. *Proc. Natl Acad. Sci. USA* **99**, 10567–10570 (2002).
149. Van Sloun, P. P. *et al.* The role of nucleotide excision repair in protecting embryonic stem cells from genotoxic effects of UV-induced DNA damage. *Nucleic Acids Res.* **27**, 3276–3282 (1999).
150. Hong, Y. & Stambrook, P. J. Restoration of an absent G1 arrest and protection from apoptosis in embryonic stem cells after ionizing radiation. *Proc. Natl Acad. Sci. USA* **101**, 14443–14448 (2004).
151. Kersemaekers, A. M. F. *et al.* Role of p53 and MDM2 in treatment response of human germ cell tumors. *J. Clin. Oncol.* **20**, 1551–1561 (2002).
152. Mayer, F. *et al.* p53 and MDM2 in germ cell cancer treatment response. *J. Clin. Oncol.* **20**, 3928–3929 (2002).
153. Masters, J. R. & Koberle, B. Curing metastatic cancer: lessons from testicular germ-cell tumours. *Nature Rev. Cancer* **3**, 517–525 (2003).
A comprehensive review on treatment sensitivity and resistance of type II GCTs. A link to embryonal characteristics of these tumours is made, and the role of p53 is questioned.
154. Rajpert-De Meyts, E. *et al.* The immunohistochemical expression pattern of Chk2, p53, $p19^{INK4d}$, MAGE-A4 and other selected antigens provides new evidence for the premeiotic origin of spermatocytic seminoma. *Histopathology* **42**, 217–226 (2003).
155. Koberle, B., Masters, J. R., Hartley, J. A. & Wood, R. D. Defective repair of cisplatin-induced DNA damage caused by reduced XPA protein in testicular germ cell tumours. *Curr. Biol.* **9**, 273–276 (1999).
156. Honecker, F. *et al.* Xeroderma pigmentosum group A protein and chemotherapy-resistamnce in human germ cell tumors. *Lab. Invest.* **83**, 1489–1495 (2003).
157. Zamble, D. B., Mikata, Y., Eng, C. H., Sandman, K. E. & Lippard, S. J. Testis-specific HMG-domain protein alters the responses of cells to cisplatin. *J. Inorg. Biochem.* **91**, 451–462 (2002).
158. Spierings, D. C., de Vries, E. G., Vellenga, E. & de Jong, S. The attractive Achilles heel of germ cell tumours: an inherent sensitivity to apoptosis-inducing stimuli. *J. Pathol.* **200**, 137–148 (2003).
159. Schenk, P. W. *et al.* Resistance to platinum-containing chemotherapy in testicular germ cell tumours is associated with downregulation of the protein kinase SRPK1. *Neoplasia* **6**, 297–301 (2004).
160. International Germ Cell Consensus Classification: a prognostic factor- based staging system for metastatic germ cell cancers. International Germ Cell Cancer Collaborative Group. *J. Clin. Oncol.* **15**, 594–603 (1997).
161. Bokemeyer, C. *et al.* Early prediction of treatment response to high-dose salvage chemotherapy in patients with relapsed germ cell cancer using [^{18}F]FDG PET. *Br. J. Cancer* **86**, 506–511 (2002).
162. Bartkova, J., Thullberg, M., Rajpert-De Meyts, E., Skakkebaek, N. E. & Bartek, J. Cell cycle regulators in testicular cancer: loss of $p18^{INK4C}$ marks progression from carcinoma *in situ* to invasive germ cell tumours. *Int. J. Cancer* **85**, 370–375 (2000).
163. Fan, S. *et al.* Cells lacking CIP1/WAF1 genes exhibit preferential sensitivity to cisplatin and nitrogen mustard. *Oncogene* **14**, 2127–2136 (1997).
164. Oosterhuis, J. W. *et al.* Effects of multiple-drug chemotherapy (*cis*-diammine- dichloroplatinum, bleomycin, and vinblastine) on the maturation of retroperitoneal lymph node metastases of nonseminomatous germ cell tumors of the testis. *Cancer* **51**, 408–416 (1983).
165. Mayer, F. *et al.* Microsatellite instability of germ cell tumors is associated with resistance to systemic treatement. *Cancer Res.* **62**, 2758–2760 (2002).
166. Mueller, T. *et al.* Failure of activation of caspase-9 induces a higher threshold for apoptosis and cisplatin resistance in testicular cancer. *Cancer Res.* **63**, 513–521 (2003).
167. Mayer, F. *et al.* Molecular determinants of treatment response in human germ cell tumors. *Clin. Cancer Res.* **9**, 767–773 (2003).
168. Timmer-Bosscha, H., de Vries, E. G., Meijer, C., Oosterhuis, J. W. & Mulder, N. H. Differential effects of all-*trans*-retinoic acid, docosahexaenoic acid, and hexadecylphosphocholine on cisplatin-induced cytotoxicity and apoptosis in a cisplantin-sensitive and resistant human embryonal carcinoma cell line. *Cancer Chemother. Pharmacol.* **41**, 469–476 (1998).
169. Bartkova, J., Thullberg, M., Rajpert-De Meyts, E., Skakkebaek, N. E. & Bartek, J. Lack of $p19^{INK4d}$ in human testicular germ-cell tumours contrasts with high expression during normal spermatogenesis. *Oncogene* **19**, 4146–4150 (2000).
170. Bartkova, J., Rajpert-De Meyts, E., Skakkebæk, N. E., Lukas, J. & Bartek, J. Deregulation of the G1/S-phase control in human testicular germ cell tumours. *APMIS* **111**, 252–265 (2003).
171. Cummings, O. W., Ulbright, T. M., Eble, J. N. & Roth, L. M. Spermatocytic seminoma: an immunohistochemical study. *Hum. Pathol.* **25**, 54–59 (1994).
172. Eble, J. N. Spermatocytic seminoma. *Hum. Pathol.* **25**, 1035–1042 (1994).
173. Chung, P. W. *et al.* Spermatocytic seminoma: a review. *Eur. Urol.* **45**, 495–498 (2004).
174. Dekker, I., Rozeboom, T., Delemarre, J., Dam, A. & Oosterhuis, J. W. Placental-like alkaline phosphatase and DNA flow cytometry in spermatocytic seminoma. *Cancer* **69**, 993–996 (1992).
175. Stoop, H. *et al.* Reactivity of germ cell maturation stage-specific markers in spermatocytic seminoma: diagnostic and etiological implications. *Lab. Invest.* **81**, 919–928 (2001).
176. Rajpert-De Meyts, E. *et al.* The immunohistochemical expression pattern of Chk2, p53, $p19^{INK4d}$, MAGE-A4 and other selected antigens provides new evidence for the premeiotic origin of spermatocytic seminoma. *Histopathology* **42**, 217–226 (2003).
177. Rosenberg, C. *et al.* Chromosomal constitution of human spermatocytic seminomas: comparative genomic hybridization supported by conventional and interphase cytogenetics. *Genes Chromosom. Cancer* **23**, 286–291 (1998).
178. Looijenga, L. H. J. *et al.* Seminomas of the canine testis; counterpart of spermatocytic seminoma of men? *Lab. Invest.* **71**, 490–496 (1994).
179. Subramaniam, K. & Seydoux, G. Dedifferentiation of primary spermatocytes into germ cell tumors in *C. elegans* lacking the pumilio-like protein PUF-8. *Curr. Biol.* **13**, 134–139 (2003).
180. Rosenberg, C. *et al.* Chromosomal constitution of human spermatocytic seminomas: comparative genomic hybridization suppored by conventional and interphase cytogenetics. *Genes Chromosom. Cancer* **23**, 286–291 (1998).
181. Verkerk, A. J. *et al.* Unique expression patterns of H19 in human testicular cancers of different etiology. *Oncogene* **14**, 95–107 (1997).
182. Durcova-Hills, G., Burgoyne, P. & McLaren, A. Analysis of sex differences in EGC imprinting. *Dev. Biol.* **268**, 105–110 (2004).
183. Hajkova, P. *et al.* Epigenetic reprogramming in mouse primordial germ cells. *Mech. Dev.* **117**, 15–23 (2002).
184. Wilkins, J. F. & Haig, D. What good is genomic imprinting: the function of parent-specific gene expression. *Nature Rev. Genet.* **4**, 359–368 (2003).
185. Walter, J. & Paulsen, M. Imprinting and disease. *Semin. Cell Dev. Biol.* **14**, 101–110 (2003).
186. Allegrucci, C., Denning, C., Priddle, H. & Young, L. Stem-cell consequences of embryo epigenetic defects. *Lancet* **364**, 206–208 (2004).
187. McGrath, J. & Solter, D. Nuclear transplantation in mouse embryos. *J. Exp. Zool.* **228**, 355–362 (1983).
The functional difference between some of the parental chromosomes, now known as genomic imprinting, was demonstrated by exchanging haploid sets of maternal and paternal chromosomes in mouse zygotyes. It was shown that a bimaternal imprinting (gynogenote) pattern favours somatic differentiation, and a bipaternal pattern favours trophoblastic differentiation.
188. Surani, M. A., Barton, S. C. & Norris, M. L. Nuclear transplantation in the mouse: heritable differences between parental genomes after activation of the embryonic genome. *Cell* **45**, 127–136 (1986).
189. Oosterhuis, J. W. & Looijenga, L. H. Current views on the pathogenesis of testicular germ cell tumours and perspectives for future research: highlights of the 5th Copenhagen Workshop on Carcinoma *In Situ* and Cancer of the Testis. *APMIS* **111**, 280–289 (2003).
190. Bredael, J. J., Vugrin, D. & Whitmore, W. F. J. Autopsy findings in 154 patients with germ cell tumors of the testis. *Cancer* **50**, 548–551 (1982).

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Competing interests statement

The authors declare no competing financial interests.

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DATABASES

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Genomic imprinting:

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