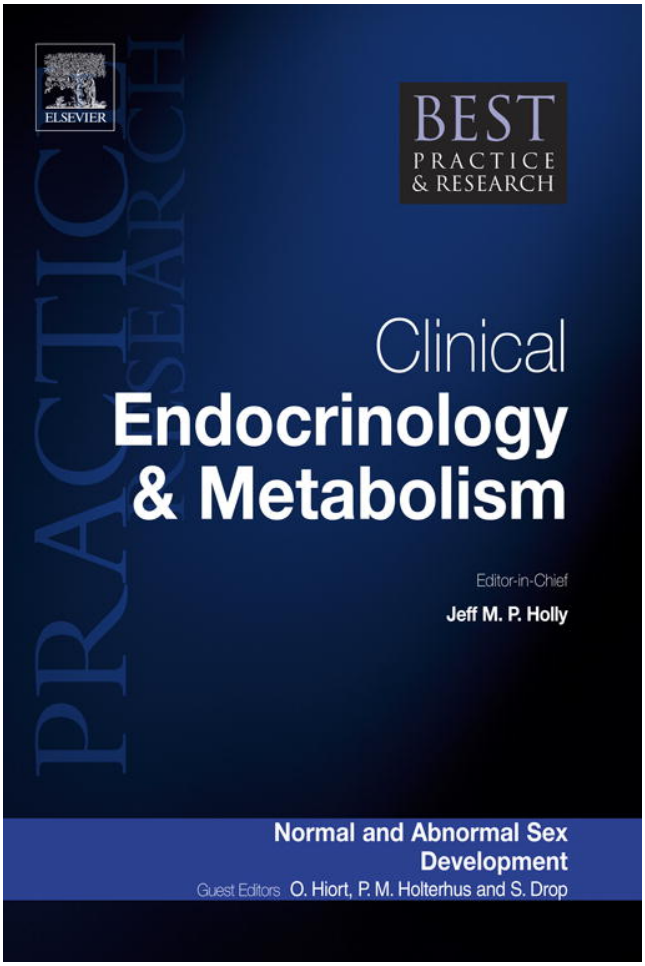

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# Tumor risk in disorders of sex development (DSD)

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Disorders of sex development (DSD), previously referred to as intersex disorders, comprise a variety of anomalies defined by congenital conditions in which chromosomal, gonadal, or anatomical sex is atypical. Besides issues such as gender assignment, clinical and diagnostic evaluation, surgical and psychosocial management, and sex steroid replacement, the significantly increased risk for developing specific types of malignancies is both clinically and biologically relevant. This relates to germ-cell tumors specifically in DSD patients with hypovirilization or gonadal dysgenesis. The presence of a well-defined part of the Y chromosome (known as the GBY region) is a prerequisite for malignant transformation, for which the testis-specific protein on the Y chromosome (*TSPY*) is a likely candidate gene. The precursor lesions of these cancers are carcinoma in situ (CIS)/intratubular germ-cell neoplasia unclassified (ITGCNU) in testicular tissue and gonadoblastoma in those without obvious testicular differentiation. Most recently, undifferentiated gonadal tissue (UGT) has been identified as the likely precursor for gonadoblastoma. The availability of markers for the different developmental stages of germ cells allows detailed investigation of the characteristics of normal and (pre)malignant germ cells. Although informative in a diagnostic setting for adult male patients, these markers – such as OCT3/4 – cannot easily distinguish (pre)malignant germ cells from germ cells showing delayed maturation. This latter phenomenon is frequently found in gonads of DSD patients, and may be related to the risk of malignant transformation. Thus, the mere application of these markers might result in over-diagnosis and unnecessary gonadectomy. It is proposed that morphological and histological evaluation of gonadal tissue, in combination with OCT3/4 and TSPY double immunohistochemistry and clinical parameters, is most informative in estimating the risk for germ-cell tumor development in the individual patient, and might in future be used to develop a decision tree for optimal management of patients with DSD.

**Key words:** germ-cell development; malignant transformation; CIS/ITGCNU; gonadoblastoma; cancer risk; delayed maturation; diagnostic markers; prediction model.

Patients with specific forms of disorders of sex development (DSD), as defined recently in the Consensus Statement on Management of Intersex Disorders[1], have an increased risk for development of cancers originating from the germ-cell lineage, also known as germ-cell tumors (GCTs). Within this group of tumors, various entities have been identified which are characterized by age at clinical presentation, histology, clinical behavior, and genomic constitution.[2] Based on these parameters, we proposed a novel classification system in which five types of GCTs are distinguished. These are summarized in Table 1, and are also recognized by the World Heath Organization.[3] In the context of DSD, only the type-II GCTs are of relevance; these are the seminomatous and non-seminomatous types of tumor. In the adult testis, these cancers are the most frequent malignancy in Caucasian males of the age group 15–45 years, and are showing an increasing incidence.[4,5] The testicular dysgenesis syndrome (TDS) has been suggested to be the underlying reason for the development of this type of testicular cancer.[6] TDS links various clinical observations, such as cryptorchidism, subfertility/infertility, and an increased risk of cancer. These features have been suggested to be the result of the same underlying mechanism. They are related to a suboptimal testicular development, to environmental factors (such as xeno-estrogens and anti-androgens), and possibly to genetic factors. The model of TDS is relevant in the context of the increased risk of development of type-II GCTs in patients with DSD, as initially proposed by Skakkebaek and co-workers (see below).

The type-II GCTs refer to seminomatous and non-seminomatous tumors. Histologically, the seminomatous tumors are composed of neoplastic primordial germ cells/gonocytes. The non-seminomatous GCTs, which result from reprogramming of

**Table 1.** Summary of the classification of germ cell tumors (GCTs) in five entities.

| Type | Histology | Cell of origin | Anatomical site |
|---|---|---|---|
| I | TE/YST | Embryonic GC[a] | Midline |
| II | SE/N | PGC/gonocyte[b] | Midline[d] |
| III | SS | Primary spermatocyte[c] | Testis |
| IV | DC | Parthogenote | Ovary |
| V | HM | Androgenote | Uterus |

TE, teratoma; YST, yolk-sac tumor; SE, seminomatous GCT (seminoma of the testis, dysgerminoma of the ovary and dysgenetic gonad and germinoma of the brain); N, non-seminoma (can be composed of embryonal carcinoma, choriocarcinoma); SS, spermatocytic seminoma; DC, dermoid cyst; HM, hydatidiform mole; GC, germ cell; PGC, primordial germ cell.

[a] Not proven in all cases.
[b] Erased pattern of genomic imprinting.
[c] Paternal pattern of genomic imprinting.
[d] Predominantly the male gonad.

a seminomatous precursor cell, are subdivided into the stem-cell component embryonal carcinoma (EC) and the somatic and extra-embryonic elements, i.e., teratoma (TE) and yolk-sac tumor (YST), choriocarcinoma (CH), and the germ line (see also Figure 1A, right).[2,3,7] It is of relevance to note that TE and YST can also be found in the so-called type-I GCTs, which are predominantly diagnosed in neonates and infants. However, these have a different cell of origin and chromosomal constitution.[8–11]

The precursor lesions of the type-II GCTs have been identified and characterized to a certain extent. The most important observations in the context of DSD will be discussed here. In order to understand the relevance of these findings, the normal germ-cell development will be presented first.

**Practice points**

- DSD patients have an increased risk for type-II GCTs
- Type-II GCTs refer to the seminomatous and non-seminomatous GCTs

## NORMAL GERM-CELL DEVELOPMENT

During recent years our understanding of the pathobiology of type-II GCTs has increased due the availability of specific markers that characterize germ cells at different stages of their development and maturation. The process of germ-cell development is strictly organized in both time and space.[12] It starts during early intrauterine development (weeks 5–7 in humans) in the proximal epiblast[13] (see also Figure 1B). Primordial germ cells (PGCs) divide and move along the hindgut to the genital ridges.[14] At this stage they show a number of unique characteristics. After their arrival in the genital ridges, they are referred to as gonocytes, but morphologically they are still indistinguishable from PGCs. In addition, they show expression of the same genes and proteins, including alkaline phosphatase, c-KIT, and OCT3/4[15] (Figure 1C, and see below). During the subsequent steps of maturation, the germ cells lose their

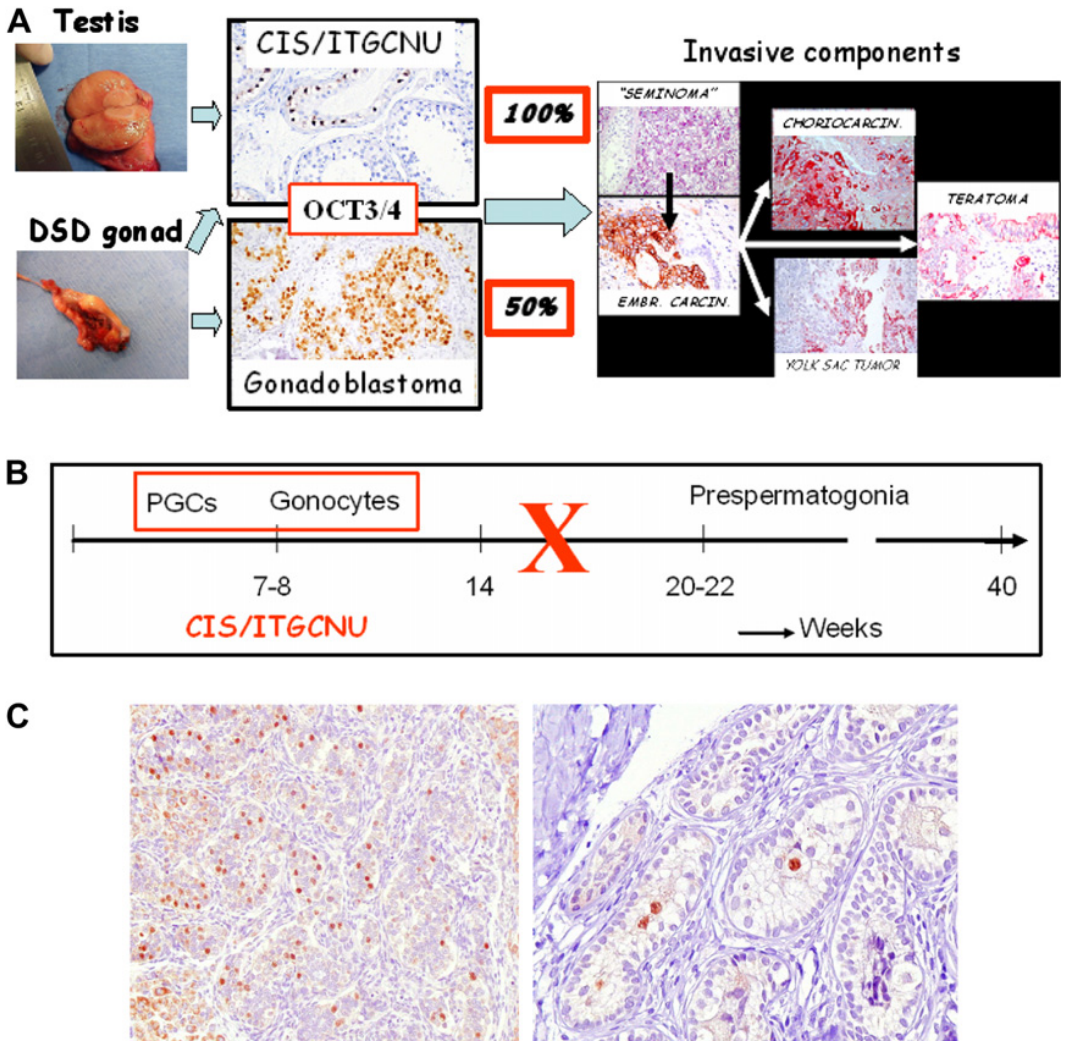


**Figure 1.** (A) Summary of the precursor lesions for the type-II germ-cell tumors (GCTs), these being carcinoma in situ/intratubular germ-cell neoplasia unclassified (CIS/ITGCNU) in the testis, and CIS/ITGCNU or gonadoblastoma in the case of dysgenetic gonads. The malignant cells can be diagnosed on the basis of immunohistochemical detection of OCT3/4, amongst other markers. These precursors will progress to invasive GCTs, with the histology of either seminoma or the different non-seminomatous elements: embryonal carcinoma, teratoma, yolk-sac tumor and choriocarcinoma. Seminomatous cells can be reprogrammed to embryonal carcinoma, the stem-cell component of the non-seminomas. Besides these histologies, the germ-cell lineage has been identified recently. All CIS/ITGCNU are supposed to form an invasive cancer, with a figure of about 50% being suggested for gonadoblastoma. (B) Schematic representation of the different developmental steps in the intrauterine generation and development of the germ-cell lineage. The CIS/ITGCNU cells mimic primordial germ cells (PGCs)/gonocytes regarding their morphology and marker expression profile, although they have an aberrant histological localization, i.e., under the tight junctions of the Sertoli cells illustrated in (A). (C) Representative examples of immunohistochemical demonstration of OCT3/4 in embryonic male gonads of 13 weeks' gestational age (left) and 1 week postpartum. Note the presence of OCT3/4-positive germ cells, with numbers declining at higher gestational age. The OCT3/4-positive germ cells after partus are located in the lumen of the seminiferous tubules, sometimes misdiagnosed as CIS/ITGCNU.

embryonic characteristics, such as expression of OCT3/4. Initially OCT3/4 was identified as one of the regulatory elements in the differentiation of embryonic stem cells[16], but OCT3/4 expression is also present in PGCs and gonocytes and their malignant counterparts (see below). This may account for the capacity of early PGCs to generate pluripotent stem cells[2] (see also below).

The timely and adequate expression of the *SRY* gene, located on Yp, will induce a cascade of reactions resulting in the expression of several genes, mostly transcription factors, and ultimately resulting in testicular development of the gonad in a male direction.[17] Due to interaction with the pre-Sertoli cells, the gonocytes differentiate into pre-spermatogonia, and as part of that process they move to the basal lamina, after which they mature further (into spermatogonia). During this maturation process they lose expression of the embryonic genes, and start enhanced expression of additional genes – amongst others VASA (Mvh) and TSPY[18] – indicative of further differentiation along the male germ-cell lineage. Differences in this process have been identified between mice and men.[19] For example, c-KIT and OCT3/4 are still expressed in mouse spermatogonia, while OCT3/4 is absent in the human counterpart, and c-KIT is immunohistochemically undetectable. In the absence of *SRY* expression, the gonad will develop in the female direction, this being the default pathway. Germ cells will form oocytes, which are blocked in their meiotic division.[20] As part of that process, they also lose expression of the aforementioned genes, including OCT3/4.[21,22]

In addition to these morphological changes, the germ cells also undergo a unique modification known as genomic imprinting.[23,24] This is the difference in functionality between a haploid set of mammalian chromosomes, depending on the parental origin. This modification is determined by epigenetic modifications such as DNA methylation. The status of genomic imprinting is informative in distinguishing the various stages of germ-cell development. The genomic imprinting pattern of PGCs/gonocytes is erased[25], while parental imprinting is established at later developmental stages of the male germ-cell lineage.[26]

**Practice points**

- germ cells undergo specific maturation stages recognizable by morphology and expression profile of genes and proteins as well as status of genomic imprinting
- OCT3/4 is a marker for primordial germ cells/gonocytes

## PRECURSOR LESIONS OF TYPE-II GCTS

The precursor of the type-II GCT of the testis is known as carcinoma in situ (CIS)/intratubular germ-cell neoplasia unclassified (ITGCNU), initially linked to the development of invasive type-II GCTs by Niels Skakkebaek[3,27]; it is also known as testicular intratubular neoplasia (TIN).[28] These cells show major characteristics of PGCs/gonocytes (see Figure 1), including their expression profile of genes and proteins such as c-KIT, PLAP and OCT3/4 (see Figure 1A, left).[29] In CIS/ITGCNU, OCT3/4 protein is found in germ cells located at the basal lamina and under the tight junctions between the Sertoli cells, whereas these specific features are not present under physiological conditions. Of note is that in mouse PGCs OCT3/4 does not regulate the lineages of differentiation, as reported in embryonic stem cells, both mouse and human[16,30], but it inhibits induction of apoptosis.[31] It is tempting to speculate that this mechanism also is operative in CIS/ITGCNU as well as seminoma cells, which are also OCT3/4-positive.[32–35] So far, in our hands, OCT3/4 is the most informative diagnostic marker for CIS/ITGCNU and gonadoblastoma (see below), and for seminoma and embryonal

carcinoma of the adult testis. However, the application of this marker in postnatal prepubertal gonads with possible delayed maturation of germ cells has to be interpreted with caution, as will be discussed below.

In gonads of DSD patients, the precursor of the cancer might be CIS/ITGCNU or gonadoblastoma, depending on the level of testicular differentiation.[36–38] Gonadoblastoma is a lesion composed of a mixture of germ cells at different stages of maturation, and of supportive pre-Sertoli/granulosa cells.[37,39,40] At least some of the germ cells are positive for OCT3/4, as are all the early invasive cells of seminoma (referred to as dysgerminoma in dysgenetic gonads). These observations support the generally accepted model that the precursor cells of type-II GCTs, both in the testis and in gonads of DSD patients, are germ cells that are blocked in the early stages of development, i.e., PGCs/gonocytes. The non-physiological histological position of these OCT3/4-positive CIS/ITGCNU cells (see above) while expressing other embryonic markers, such as NANOG[41,42], at the basal lamina, under the tight junctions between the Sertoli cells, is an interesting observation, the impact of which has not yet been elucidated. On the basis of the extrapolation of epidemiological data, it is expected that all patients with CIS/ITGCNU, and a suggested 50% with gonadoblastoma, will eventually develop invasive type-II GCTs.[43,44] These estimations are not based on empirical data. Most recently, detailed histological investigation of gonads of DSD patients led to the identification of the precursor of gonadoblastoma, which was referred to as undifferentiated gonadal tissue (UGT).[45] Further characterization of these lesions will deepen our knowledge about the earliest stages in the pathogenesis of type-II GCTs.

**Practice points**

- the precursor of type-II GCTs in the testis is CIS/ITGCNU
- the precursor lesion of type-II GCTs in the dysgenetic gonad can be CIS/ITGCNU or gonadoblastoma
  OCT3/4 is positive in the malignant cells of these precursors

## DELAY IN MATURATION AND MALIGNANT TRANSFORMATION: A DIAGNOSTIC DILEMMA IN DSD

As indicated above, the most frequent precursor lesion of type-II GCTs is CIS/ITGCNU, consisting of germ cells blocked in their physiological process of maturation, and positioned at an abnormal localization. This observation has important implications. It means that in fact no specific markers are available to distinguish germ cells that are delayed in their maturation from those undergoing malignant transformation. This limits the application of these diagnostic markers in cases in which delayed germ-cell maturation can be expected. This might result in an incorrect diagnosis of CIS/ITGCNU and gonadoblastoma, resulting in over-treatment by gonadectomy, especially in young patients. The current parameters used (by us) to diagnose malignant germ cells in gonads of patients with hypovirilization are summarized in Table 2.[36,46] Using these criteria, a pre-CIS/ITGCNU has been identified in two patients, one with partial androgen insensitivity and one with gonadal dysgenesis. Characterization of these cells is a next step in the elucidation of the earliest stages of the process of type-II GCT development.

**Table 2.** Additional criteria for the diagnosis of maturation delay and carcinoma in situ/intratubular germ-cell neoplasia unclassified (CIS/ITGCNU) in XY individuals with under-virilization syndrome.

| | Maturation delay | Transition | CIS |
|---|---|---|---|
| Patient age | <1 year | Prepubertal | >1 year |
| Position of OCT3/4$^+$ cells within the seminiferous tubule | Luminally | Luminally and on the basal lamina | On the basal lamina |
| Position of OCT3/4$^+$ cells throughout the gonad | Widespread | Confined to a specific region; the rest of the gonad is free of positive cells or displays characteristics of maturation delay | Confined to a specific region; the rest of the gonad is free of positive cells or displays characteristics of maturation delay |

It is of interest that a marked delay in germ-cell maturation is found only in fetal gonadal tissue of male trisomy 21 patients and not in females.[47] This might explain the higher incidence of type-II GCTs in male trisomy 21 patients. In other words, the identification of a new marker allowing unambiguous distinction between malignant germ cells and germ cells delayed in their maturation might ultimately result in a better insight into the individual risk of developing a type-II GCT and hence perhaps in a more risk-adapted treatment of patients with DSD. Various studies are currently being undertaken to identify such a marker. In particular the role of the Y chromosome in normal and malignant development is highly relevant and will be discussed below.

**Practice points**

- germ-cell maturation delay is a frequent finding in patients with DSD
- maturation-delayed germ cells cannot easily be distinguished from malignant germ cells

## THE ROLE OF OCT3/4 AND THE Y CHROMOSOME IN TYPE-II GCTS

The 'simple' observation that type-II GCTs are the most frequent malignancies of the testis in young adult Caucasian males links their development to male gonadal differentiation and therefore to the Y chromosome. Interestingly, patients with Klinefelter syndrome (47,XXY) have no increased risk for testicular type-II GCTs, but they do for similar types of cancer of the mediastinal/thymus region.[48] This might be related to the fact that testicular germ cells disappear in XXY gonads, most likely because of an improper microenvironment.[49] The same may apply for the gonads of patients with complete androgen insensitivity.[36] Based on studies in patients with gonadoblastoma or an invasive tumor, in whom Y chromosomal material has been identified in molecular studies, it was concluded that a specific region of the Y chromosome is crucial for

development of this type of cancer.[50] It was found that the region around the centromere of the Y chromosome – referred to as the GBY region – is of importance.[51,52] A number of candidate genes are located in this chromosomal fragment, including *TSPY*, which stands for testis-specific protein on the Y chromosome.[37,53–56] This multicopy gene[57–59] is expressed in germ cells.[53] More interesting is the finding that it is present in large amounts in CIS/ITGCNU and gonadoblastoma cells[37] (see Figure 2A); the mechanism for this is unclear so far. A transgenic mouse model has been generated, in which the human gene is integrated in multiple copies on the Y chromosome. However, up to adult age there is no specific phenotype.[60] Most of the precursor cells of the type-II GCTs show a double-positive staining for TSPY and OCT3/4 (see Figure 2A). It is expected that TSPY is involved in regulation of proliferation.[56,61] This is also based on biochemical studies as well as deducted from structural characteristics. Based on these data, an interesting model is emerging: it is the combination of inhibition of apoptosis, under control of OCT3/4, and induction of proliferation, under control of TSPY (see Figure 2B)[37], that results in malignant transformation of embryonic germ cells, leading to CIS/ITGCNU and gonadoblastoma.

An interesting recent observation is that although SRY induces the first step in testicular differentiation during embryogenesis, the relationship between the quantitative presence of the Y chromosome (determined by in-situ hybridization techniques) and testicular histology is not simple (Cools et al, submitted for publication). It remains to be determined whether this is related to the functionality of the Y chromosome, i.e., to induction of the SRY-induced signaling cascade.

**Practice points**

- presence of the GBY region is crucial for development of type-II GCTs
- TSPY is a likely candidate gene for the GBY region

## ADDITIONAL MALIGNANT 'INTRINSIC' CHARACTERISTICS OF EMBRYONIC GERM CELLS

It is of interest that PGCs/gonocytes have a number of characteristics in common with type-II GCTs, which are represented in the phenotype of cells undergoing malignant transformation (see Figure 3A).[62] These include the presence of telomerase activity[63–67] and an erased pattern of genomic imprinting[68–72], which has been related to tumorigenesis in the mouse[73], including development of a testicular seminoma. This has never been reported in any animal so far.

One of the unsolved issues in the pathogenesis of type-II GCTs is the overall presence of wild-type P53 protein[74–76], the most frequently inactivated gene in solid cancers.[77] The requirement to inactivate P53 is (partly) due to the role the P53 protein plays in overruling the mechanism of cellular senescence, induced for example by oncogenic stress.[78] This discrepancy has recently been elucidated by our showing that a specific set of microRNAs (i.e. hsa-miR 371–373) is expressed in seminomas and the undifferentiated component of non-seminomas, in the presence of wild-type P53.[79] Expression of these microRNAs interferes with the process of translation of the tumor suppressor *LATS2* mRNA, which under physiological conditions blocks the G1–S transition in the cell cycle. In the case of a mutation in P53, which is a very rare phenomenon in type-II GCTs, the miRNA cluster 371–373 was not

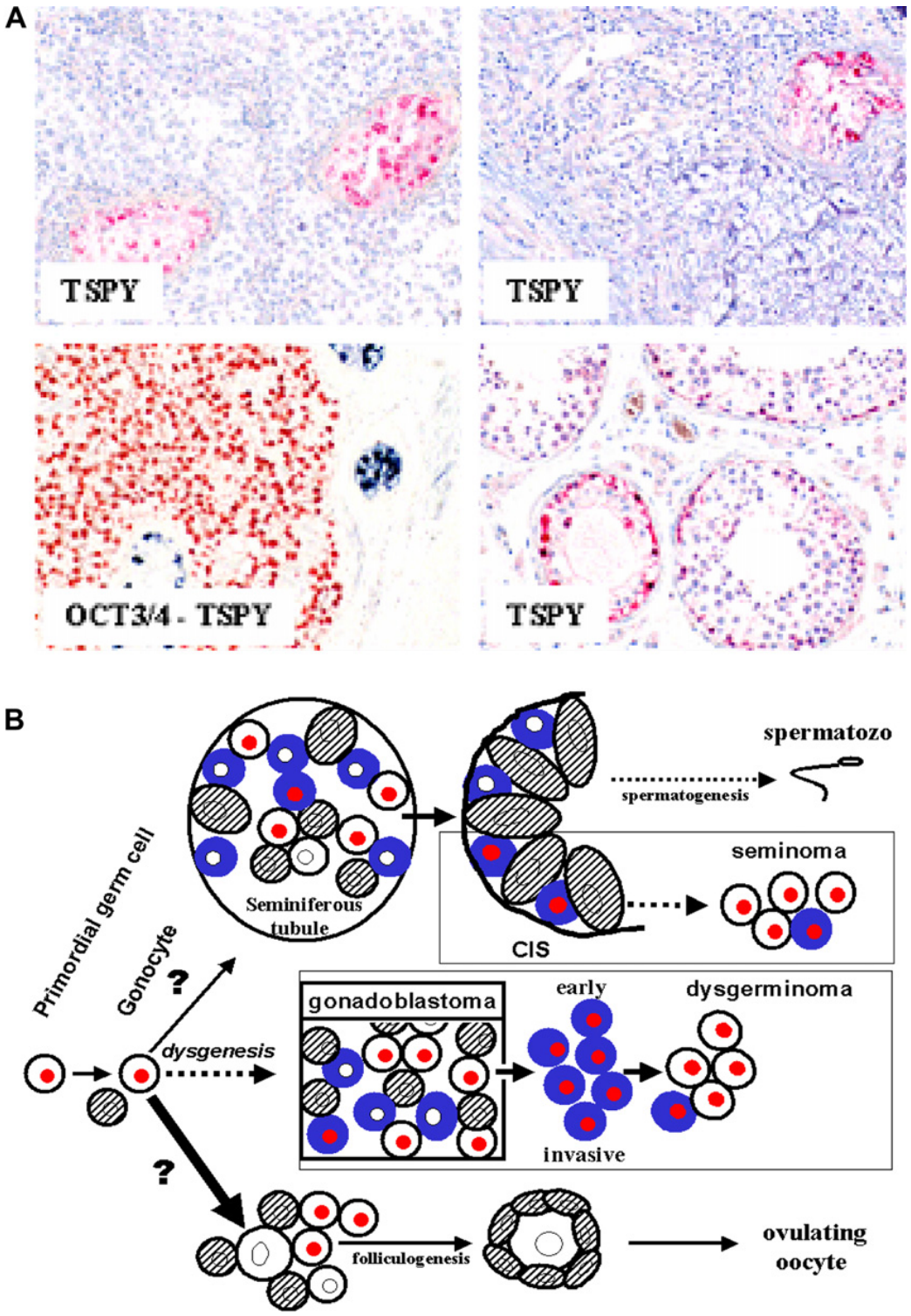


**Figure 2.** (A) Representative examples of immunohistochemical demonstration of TSPY (red staining) on carcinoma in situ/intratubular germ-cell neoplasia unclassified (CIS/ITGCNU) and seminoma (upper left panel) and embryonal carcinoma (upper right panel), as well as combined with OCT3/4 on CIS/ITGCNU and seminoma (lower left panel) (TSPY = blue; OCT3/4 = red), and on CIS/ITGCNU-containing testicular parenchyma (lower right panel). Note the more intense TSPY staining in CIS/ITGCNU compared to normal germ cells. (B) Schematic representation of the pathogenetic model of type-II germ-cell tumors (GCTs) in the testis and dysgenetic gonad, in which OCT3/4 (indicated by a red nucleus: inhibition of apoptosis) and TSPY (indicated by a blue cytoplasm: induction of proliferation) are involved. Note that the double-positive germ cells are the cells that give rise to the invasive cancer. TSPY is frequently lost upon invasive growth.[37]

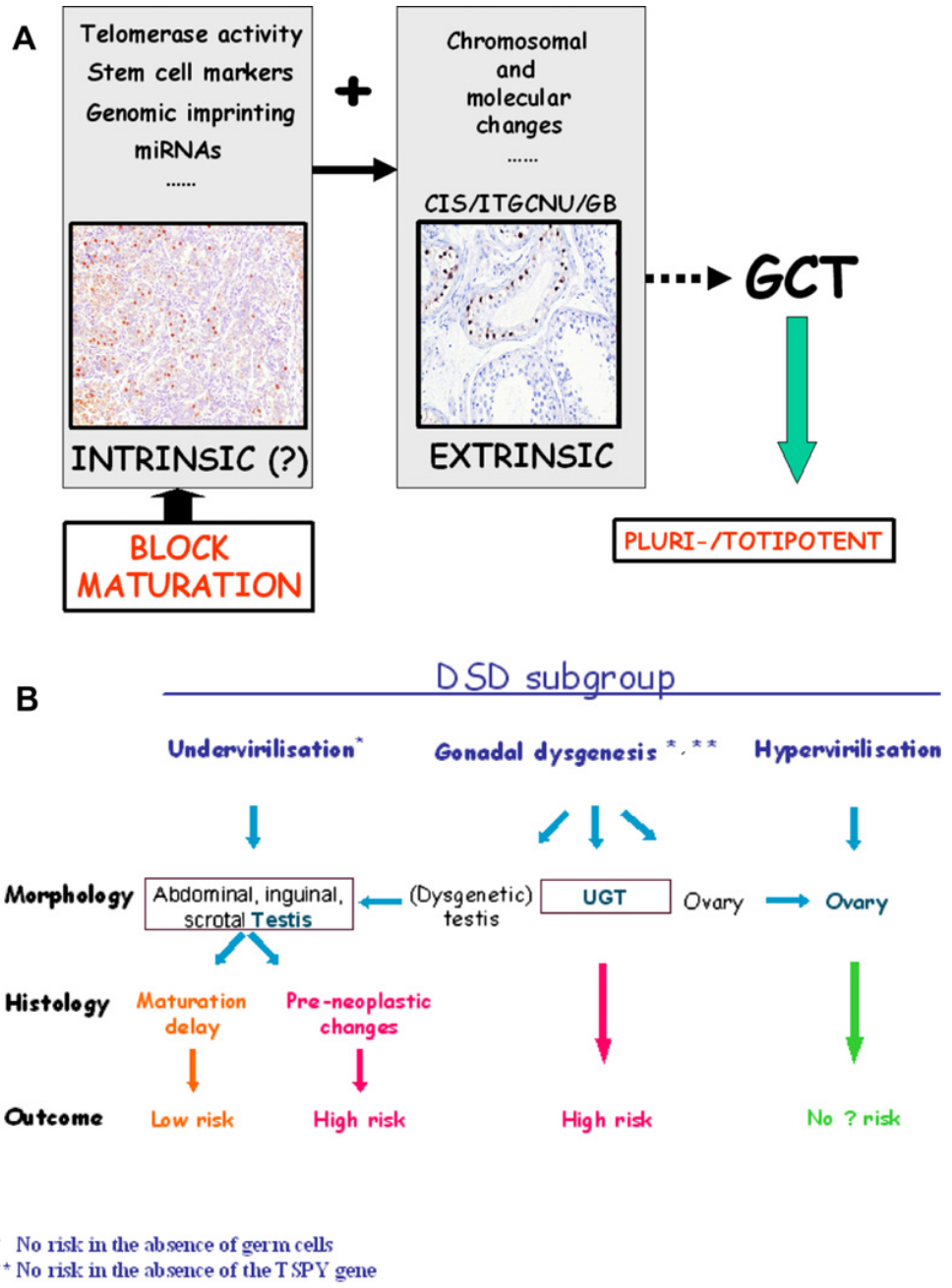


**Figure 3.** (A) Summary of the different (intrinsic?) characteristics of embryonic germ cells (primordial germ cells/gonocytes), i.e., telomerase activity, expression of stem-cell markers, erased pattern of genomic imprinting, and expression of specific miRNAs, that are also reported to be related to the process of malignant transformation. Due to the block in differentiation of these germ cells, these characteristics are retained in carcinoma in situ/intratubular germ-cell neoplasia unclassified (CIS/ITGCNU) and gonadoblastoma (GB). These precursor cells will progress to invasive tumors. (B) Schematic representation of the prediction tree for development of type-II germ-cell tumors (GCTs) in patients with disorders of sex development (DSD). The parameters to be investigated are morphology and histology of the gonadal tissue in the context of the clinical findings of under-virilization, over-virilization, and gonadal dysgenesis. Proper distinction between delayed maturation and malignant transformation is of crucial importance. The presence of the GBY region is represented by analysis of the TSPY protein.

expressed. This was observed both in tumors in vivo as well as in tumor-derived cell lines.

**Practice point**

- embryonic germ cells show characteristics of malignant cells

## TUMOR RISK IN DIFFERENT SUBGROUPS OF DSD

Although overall DSD patients have an increased risk for development of type-II GCTs, this risk is significantly different in the clinical subgroups. This has been discussed in detail elsewhere[1,38], but the major findings will be summarized here. For better understanding, the following definitions are used.

- *Gonadal dysgenesis*: an incomplete or defective formation of the gonads, mostly due to a disturbed process of migration of the germ cells and/or their correct organization in the fetal gonadal ridge. This is due to structural or numerical anomalies of the sex chromosomes or mutations in genes involved in the formation of the urogenital ridge and in sex determination of the bipotential gonad.[38]
- *Hypervirilization* refers to 46,XX individuals who are exposed to androgens – of endogenous (due to genetic defects in enzymes involved in adrenal steroid hormone production) or exogenous origin – during fetal life.
- *Under-virilization* refers to incomplete masculinization or the ambiguous or female phenotype in an XY individual due to errors in testosterone biosynthesis or action in androgen-dependent target tissues and unresponsiveness to stimulation from the pituitary, or to defects in androgen-dependent target tissues.

The risk for type-II GCT development in the patients with DSD can be classified into different levels: high, intermediate, low and unknown (see Table 3). It is clear that for a number of entities it is based on a limited number of studies, sometimes including small numbers of patients. Therefore, additional studies need to be performed before a final conclusion can be drawn. In spite of this limitation, several statements can already be made. At high risk are patients with gonadal dysgenesis with the GBY region in their genome and intra-abdominal gonads, patients with partial androgen insensitivity syndrome with non-scrotal gonads, and patients with Frasier and Denys–Drash syndromes. The percentages found in the literature vary from 15 to 60%. At intermediate risk are patients with the $Y^{+}$ ($GBY^{+}$) Turner syndrome and those with 17β-hydroxysteroid dehydrogenase (17β-HSD) deficiency, gonadal dysgenesis (including the Y chromosome), or partial androgen insensitivity, the two latter categories with scrotal gonads. The low-risk group includes patients with complete androgen insensitivity as well as patients with ovotestis DSD and those with Turner syndrome lacking an apparent Y chromosome in their karyotype. The unknown category includes 5α-reductase deficiency and Leydig-cell hypoplasia, for which there are insufficient data for proper analysis.

This first attempt to estimate the risk of the individual patient with DSD developing a type-II GCT must be tested using additional cases in which proper criteria are used for classifying patients in the different DSD entities, for histological characterization, determination of the presence of CIS/ITGCNU and gonadoblastoma compared to

**Table 3.** Risk of type-II germinal cell tumors (GCTs) in the various categories of disorders of sex development (DSD) patients, classified into high-, intermediate-, low- and no-risk groups.

| Risk group | Disorder | Malignancy risk (%) | Recommended action | Studies (n) | Patients (n) |
|---|---|---|---|---|---|
| High | GD[a] (+Y)[b] intra-abdominal | 15–35 | Gonadectomy[c] | 12 | >350 |
| | PAIS non-scrotal | 50 | Gonadectomy[c] | 2 | 24 |
| | Frasier | 60 | Gonadectomy[c] | 1 | 15 |
| | Denys–Drash (+Y) | 40 | Gonadectomy[c] | 1 | 5 |
| Intermediate | Turner (+Y) | 12 | Gonadectomy[c] | 11 | 43 |
| | 17β-HSD | 28 | Monitor | 2 | 7 |
| | GD (+Y)[c] | Unknown | Biopsy[d] and irradiation? | 0 | 0 |
| | PAIS scrotal gonad | Unknown | Biopsy[d] and irradiation? | 0 | 0 |
| Low | CAIS | 2 | Biopsy[d] and ??? | 2 | 55 |
| | Ovotestis DSD | 3 | Testis tissue removal? | 3 | 426 |
| | Turner (− Y) | 1 | None | 11 | 557 |
| No (?) | 5α-reductase | 0 | Unresolved | 1 | 3 |
| | Leydig cell hypoplasia | 0 | Unresolved | 2 | |

CAIS, complete androgen insensitivity syndrome; 17β-HSD, 17β-hydroxysteroid dehydrogenase deficiency; PAIS, partial androgen insensitivity syndrome.

[a] Gonadal dysgenesis (including not further specified, 46XY, 46X/46XY, mixed, partial, complete).

[b] GBY region positive, including the *TSPY* gene.

[c] At time of diagnosis.

[d] At puberty, allowing investigation of at least 30 seminiferous tubules, with diagnosis preferably based on OCT3/4 immunohistochemistry.

delayed maturation. Using the aforementioned data, as well as the characterization of the pre-malignant cells of type-II GCTs, a model is proposed to predict the risk for malignant transformation in patients with DSD. This is schematically presented in Figure 3B, and is currently being investigated for its clinical value. It is expected that this model will be modified on the basis of additional information gathered in the various national and international studies.

**Practice point**

- patients with DSD can be classified into high, intermediate, low, or unknown risk groups for type-II GCTs

## CONCLUSIONS

Increasing knowledge of the characteristics of both normal and malignant germ cells has become available. The invasive GCTs related to patients with DSD are the seminomatous and non-seminomatous tumors, referred to as type-II GCTs. These originate from precursor lesions – i.e. CIS/ITGCNU and gonadoblastoma – containing embryonic germ cells (PGC/gonocyte) blocked in their maturation. The formation of CIS/ITGCNU or gonadoblastoma depends on the level of testicular development of the gonad. The (pre)malignant cells can be identified using immunohistochemical

detection of markers for embryonic germ cells, such as OCT3/4. It must be kept in mind that these markers are not able to distinguish germ cells showing delayed maturation from those that are malignantly transformed. Therefore, additional criteria must be applied or a specific marker must be developed to prevent over-diagnosis. Based on strict clinical and histological criteria, the various entities of patients with DSD can be classified into different groups according to their risk for developing type-II GCTs, i.e., high, intermediate, low (and so far unknown) risk groups. This model must be tested, and possibly corrected after examination of additional series of well-characterized patients.

**Research agenda**

- identification of the optimal parameters for distinguishing germ cells with delayed maturation versus malignant transformation
- extensive investigation of the type-II GCT risk in the various forms of DSD
- clinical testing of proposed risk-stratification model

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