Review

Carcinoma in situ testis, the progenitor of testicular germ cell tumours: a clinical review

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Testicular germ cell tumours (TGCT), including seminomas, embryonal carcinomas, teratomas and yolk sac tumours, have a common precursor, the carcinoma in situ (CIS) cell. Recent gene expression studies displaying close similarity of CIS cells to embryonic stem cells support the long-standing theory that CIS most likely originates in utero from fetal gonocytes. The clinical association between the testicular dysgenesis syndrome components (TGCT, cryptorchidism, genital malformations, some forms of decreased spermatogenesis) also implies a prenatal origin. Despite high cure rates of TGCT, efforts should be made to obtain diagnosis at the CIS stage, as intervention is possible before an invasive tumour develops, thus reducing the necessity for intensive therapy. CIS may be suspected in patients with an assumed extragonadal GCT or cryptorchidism, and in intersex patients and selected cases with infertility (presenting with atrophic testes and ultrasonic microlithiasis). Surgical testicular biopsy seems the only reliable diagnostic method. The management of choice of unilateral CIS is orchidectomy, or localised irradiation in bilateral cases. At least 5% of TGCT patients present with contralateral CIS; therefore, contralateral biopsy is recommended at the time of orchidectomy. Further research is warranted to identify causal factors explaining the increasing incidence of TGCT and to obtain a method of non-invasive CIS detection.

Key words: intratubular germ cell neoplasia, unclassified type, testicular cancer, testicular dysgenesis syndrome, testicular intraepithelial neoplasia

Introduction

Testicular cancer is the most common neoplastic malignancy in 20- to 34-year-old males and the lifetime risk, which is ~0.5–1%, has in some countries increased up to three-fold in the last five decades [1]. More than 95% of testicular tumours are of germ cell origin [testicular germ cell tumours (TGCTs)] and can be divided into two main types: seminomas and non-seminomas. The latter type may harbour one or several components, including embryonal carcinoma, teratoma, polyembryoma, choriocarcinoma or yolk sac tumour. In ~10% of cases both seminoma and non-seminoma may develop simultaneously in one testicle as the so-called combined (or mixed) germ cell tumour [2].

Virtually all TGCTs are believed to originate from a common precursor, the carcinoma in situ (CIS) cell [3], with the exception of the rare spermatocytic seminoma occurring in elderly men and infantile tumours (yolk sac tumours and mature teratomas). Varying terms for the precursor are used, including intratubular germ cell neoplasia [4] and testicular intraepithelial neoplasia [5]. At present, very few patients are diagnosed with CIS, although CIS almost invariably progresses to overt testicular cancer. The circumstances in which CIS should be suspected are well defined, and the opportunity to intervene is available before an invasive tumour has developed.

Histopathology and biological aspects of CIS

CIS cells are large cells with distinct nucleoli, which in a typical pattern are located in a single row at the usually thickened basement membrane of seminiferous tubules, which have decreased diameters (Figure 1) [6]. The most commonly used marker in clinical practice is placental-like alkaline phosphatase [7], a tissue-specific alkaline phosphatase with unknown biological function in CIS cells (Figure 1C). The assumption that the CIS cell is the precursor of TGCTs is supported by the frequent observation of CIS in testicular parenchyma surrounding invasive cancer, as well as the development of invasive TGCTs in patients in whom CIS has previously been diagnosed. Testicular parenchyma with CIS is frequently atrophic and may contain signs of dysgenesis with incompletely differentiated tubules, poor spermatogenesis...
and microliths (microcalcifications) (Figure 1E) [8]. Prepubertal testes may occasionally harbour CIS cells in low numbers and their morphology resembles not only adult CIS cells, but also normal infantile gonocytes in many respects, which is why prepubertal CIS may be difficult to diagnose [9]. CIS cells stay quiescent during infancy followed by proliferation in puberty, probably due to hormonal stimulation, with subsequent progression into overt tumours. The spontaneous course of CIS gives an estimated risk of developing invasive growth in a testis harbouring CIS of 50% within 5 years and 70% within 7 years [10]. It is assumed that all patients harbouring CIS will develop an invasive tumour with time, although seldom cases of ‘burned out’ CIS have been reported.

Research into basic aspects of CIS has been hampered by the fact that CIS cells cannot be cultured in vitro and by a lack of a good animal model. With respect to the pathogenesis of TGCTs it is important to know how the initial malignant transformation proceeds from a precursor cell to the CIS cell, what aetiological factors stimulate this event, and how this single precursor cell can have such a variable differentiation potential, giving rise to either the germ-cell determined lineage (seminoma) or the pluripotent embryonal carcinoma, teratomas and even extra-embryonic elements, such as yolk sac tumour and choriocarcinoma. The initiation of malignant transformation most likely takes place in utero during the early development of the germline stem cell, and the target cell is probably a gonocyte. This hypothesis was initially based on morphological resemblance between CIS cells and gonocytes [11, 12], with subsequent studies demonstrating overlapping expression patterns between fetal gonocytes and CIS cells of several proteins, for example KIT [13], OCT3/4 [14] and AP-2γ [15], which are not detectable in the adult testis. The pathogenesis of CIS development (reviewed in [16]).

Figure 1. Histological and ultrasonical appearance of carcinoma in situ (CIS) testis. (A, B) Section of a testicular biopsy containing seminiferous tubules with abundant CIS (CIS arrows) and Sertoli cells (S arrows), haematoxylin–eosin staining. (C) A tubule with CIS (left) and a tubule with preserved spermatogenesis (right) are shown side by side. Only malignant CIS cells are stained red/brown with the placental alkaline phosphatase antibody [7]. (D) Nuclear staining of CIS cells with an AP-2γ antibody [15]. (E) Section of a testicular biopsy with a large intratubular microlith. (F) Ultrasound image of a testis containing CIS. Ultrasonic pattern is irregular with microlithiasis (arrows). Scale bar, 50 μm.
may be tightly connected to the expression of these and other genes. \textit{KIT}, for example, is a protooncogene involved in migration and differentiation of primordial germ cells, and it has been hypothesised that the expression of this antiapoptotic factor in developmentally delayed germ cells could contribute to the neoplastic development by prolonging their survival. OCT3/4 has been reported to maintain stem cells in the pluripotent stage and prevent differentiation, and \textit{AP-2} is a gene involved in self-renewal and survival of immature germ cells. Recently, further knowledge has been obtained by gene expression profiling studies, which have provided evidence for a stem-cell-like phenotype of CIS cells, as CIS and embryonic stem cells share expression of many genes that are of importance for fetal development [17, 18].

\textbf{Risk factors and prevalence of CIS in various patient populations}

A number of risk factors have been identified for TGCTs, many of which are prenatal and may be related to environmental influences or lifestyle at the time of early development. Among conditions associated with TGCTs are contralateral TGCT (relative risk 25), cryptorchidism (relative risk 4.8), familial testis cancer (relative risk 3–10) and gonadal dysgenesis [19]. Other risk factors are increased exposure to maternal hormones, the first birth and low birth weight [20, 21].

Information about the prevalence of CIS in the general population of young adults is not available, but has been estimated to be slightly below 1% in Denmark, i.e. the same as the lifetime risk of testicular cancer in the Danish male population [22]. In retrospective studies of infertile men, CIS was found in \(-0.5–1% [23, 24]\) and infertility has been stated as a risk factor for testicular cancer [25]. In infertile patients, the highest risk of CIS is in males with atrophic testes and extremely low sperm counts (\(<3 \times 10^6/\text{ml}\)). Patients with a one-sided testicular tumour have an increased risk of developing a second tumour, and the prevalence of contralateral CIS seems to be around 5–8%, corresponding well to the known incidence of bilateral testicular tumours [8, 26, 27]. In adult men with a history of cryptorchidism, CIS prevalence is 2–4% [28], which corresponds well to the known incidence of TGCTs in this group. There is an increased risk of CIS in patients with cryptorchidism irrespective of the age of surgical orchidopexy. Children with ambiguous genitalia and testicular tissue harbour CIS in \(>6\%\) of cases [29]. Finally, in a recent study of 46 intersexual patients, CIS (in addition to gonadoblastoma) was detected in more than half of cases with mixed or partial gonadal dysgenesis and in 16.7% of cases with true haemaphroditism [30].

\textbf{Current views on CIS pathogenesis}

The increased risk of CIS and testicular cancer in various disorders, such as cryptorchidism and infertility [19], may not be coincidental, and it has been proposed that these abnormalities, along with hypospadias, are components of the so-called testicular dysgenesis syndrome (TDS) [31]. In mild cases of TDS, the only symptom may be a decreased semen quality but in more severe cases several of the components may be present in the same patient [32]. When testes with CIS are evaluated histologically, dysgenetic features are not uncommon and these changes are also found in testicular biopsies of subjects with cryptorchidism and infertility.

A possible causal role of lifestyle or environmental factors in the aetiology is implicated by the significant rise in incidence of TGCT and other symptoms of the TDS in recent decades [1, 33–36], and the fact that epidemiologically the occurrence of one disorder is a risk factor for the occurrence of another. We assume that all components of TDS share common aetiological factors, which include environmental factors acting during development in individuals with genetic predisposition [37]. This may be due to an intrauterine or perinatal hormonal imbalance that delays differentiation of germ cells and may render them more susceptible to transformation [38]. The following malignant progression can, in early infancy or around puberty, be triggered by endocrine stimulation [31, 39]. This may explain the extremely rare occurrence of testicular tumours in individuals with hypogonadotropic hypogonadism, despite the fact that the testes are usually underdeveloped. The hormonal links have drawn attention to endocrine disrupters, chemicals widely present in the environment that interfere with hormonal pathways. The candidate chemical agents include natural and synthetic oestrogens and anti-androgens, e.g. those often found in plastics and cosmetics or components of pesticides. The two groups of hormones are known to adversely affect testicular development in animal models [34]. However, the causal link between endocrine disrupters and human reproductive disorders is much more difficult to prove, and remains a plausible hypothesis.

\textbf{Clinical diagnosis and management of CIS}

Despite the high cure rate of testicular cancer [36], efforts should be made to obtain diagnosis at the preinvasive CIS stage, as the disease is potentially lethal and treatment has severe side-effects, especially with regard to reproductive function. There is at present no imaging technique and no serological method for the diagnosis of CIS, which is usually asymptomatic. The diagnosis of CIS is established by a surgical biopsy, which should be \(\sim 3 \times 3\) mm in diameter, and should contain a minimum of 30–40 tubules upon histological cross-section to be representative of the whole testis [40]. If a biopsy is performed in this way, the diagnostic sensitivity is close to 100% [41], although some groups suggest double biopsies, owing to the fact that some false-negative biopsies have been reported [27]. The performance of a testicular biopsy poses a 3% risk of developing oedema, superficial haematoma or infection, complications being more likely to occur in small testes and following orchidopexy [27].

Performing a contralateral testicular biopsy at the time of orchidectomy remains a contentious issue, where some groups
Selected subfertile patients with either low semen quality and/or hypotrophic testes, especially those with an irregular ultrasound pattern including microoliths, have a history of cryptorchidism, very low sperm concentration and/or hypotrophic testes, especially those with an irregular ultrasound pattern including microoliths.

Table 1. General recommendations on screening and treatment of carcinoma in situ (CIS) testis (see text for details)

<table>
<thead>
<tr>
<th>Screening for CIS should be performed in</th>
<th>Screening modality</th>
<th>Treatment of CIS</th>
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<tbody>
<tr>
<td>Patients with a unilateral testis cancer</td>
<td>Clinical examination, testicular ultrasound and biopsy of the contralateral testis</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>Patients with presumed extragonadal germ cell tumour</td>
<td>Clinical examination, testicular ultrasound and biopsies of both testes</td>
<td>Orchidectomy if unilateral, radiotherapy if bilateral</td>
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<tr>
<td>Patients with somatosexual ambiguity and a Y chromosome</td>
<td>Clinical examination, testicular ultrasound and biopsies of both gonads</td>
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Screening for CIS should be performed in patients in whom a contralateral biopsy has not been performed in the patient groups listed in Table 1. Management of CIS prevents progression to an overt TGCT and possible metastasis, and may either be orchidectomy, radiotherapy or, in seldom cases, surveillance. The treatment modality depends mainly on the age of the patient and whether the neoplasia is unilateral or bilateral. If CIS-like cells are detected in the testis of a prepubertal boy, therapy must be individualised, as prepubertal CIS may be difficult to acknowledge histologically [44] and the natural history of prepubertal CIS has not been fully elucidated [9, 45]. This could be the case in a cryptorchid testis, where the biopsy may be delayed until after puberty, and the further management then planned if an adult CIS pattern is confirmed. Treatment may be delayed, with careful surveillance, in patients with CIS who wish to father a child, as there usually is a relatively long time-span between the diagnosis of CIS and the development of an overt TGCT. Whatever the reason for the delay, the patient should be offered semen cryopreservation when CIS has been diagnosed.

If unilateral CIS is detected in an adult with a well-functioning contralateral testis, orchidectomy is recommended as the sole treatment [46]. The testis harbouring CIS often has a very sparse spermatogenesis, and androgen production is often sufficient by the remaining testis. In contralateral CIS accompanying a testicular tumour and in the rare cases of bilateral CIS, local low-dose radiotherapy (dose level: 2 Gy × 7–10) has been shown to eradicate CIS [47, 48]. The rationale for this procedure is to destroy the dividing CIS cells and germ cells, and to preserve the non-dividing and more radioresistant Leydig cells and the external shape of the testis. Follow-up testicular biopsies after radiotherapy reveal a Sertoli-cell-only pattern, and Leydig cell function will be at least partially preserved [48]. Occurrence of a germ cell cancer despite previous local radiotherapy has been reported in a few cases [48]. Cisplatin-based chemotherapy may in some cases eradicate CIS, but persistence of CIS or subsequent development of a tumour has been observed, which is why additional irradiation or follow-up biopsies is usually recommended [49].

A staging procedure including X-ray of the chest, computed tomography scan of the abdomen and measurement of serum tumour markers (alpha-fetoprotein and ß-human chorionic gonadotrophin) should be performed in patients with CIS, even without an overt tumour. This maps the extent of the pathology and is imperative, because testicular CIS may accompany up to one-third of cases with an extragonadal GCT, especially those with a retroperitoneal tumour [50]. In patients with CIS, pretreatment fertility is usually poor [51], and cryopreservation of semen should always be considered prior to initiation of treatment, as CIS and testicular cancer often strikes young men who have not yet started or completed a family. Follow-up after the treatment of CIS should include determination of serum testosterone levels, and replacement therapy be offered in patients with subnormal levels.

Concluding remarks

Since it was first stated in 1972 that TGCTs arise from CIS cells, research has provided several pieces of new knowledge.
on basic and clinical aspects of this precursor lesion. The clinical advances enable testicular cancer to be diagnosed at the preinvasive CIS stage in at-risk patients, based on careful clinical examination combined with ultrasonography and a surgical biopsy. Regarding the pathogenesis, the hypothesis of the fetal origin of CIS and hence testicular cancer has been underlined by data showing that CIS cells have gonocyte and embryonic stem-cell characteristics. However, in spite of the progress, we still do not have firm evidence regarding the disease’s aetiology. Our current hypothesis is that environmental factors, perhaps combined with a genetic predisposition, are involved in the origin of CIS.

In light of the recent marked increase in incidence of testicular cancer, further research is warranted to identify causal factors that would explain this rise and perhaps lead to a change in the trends. Furthermore, it would be valuable to obtain a method of non-invasive detection of CIS, thus rendering obsolete the continuous discussion on the usefulness of the surgical biopsy. Whether or not such a method becomes available soon, and in spite of the high cure rate of testicular cancer, efforts should be made to establish diagnosis at the preinvasive stage, as the diagnosis of CIS is not of mere academic interest, but makes it possible to offer the patient optimal, evidence-based treatment.

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References

31. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 2001; 16: 972–978.