

Diagnostic markers for germ cell neoplasms: from placental-like alkaline phosphatase to micro-RNAs

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Abstract

This concise review summarises tissue and serum markers useful for differential diagnosis of germ cell tumours (GCT), with focus on the most common testicular GCT (TGCT). GCT are characterised by phenotypic heterogeneity due to largely retained embryonic pluripotency and aberrant somatic differentiation. TGCT that occur in young men are divided into two main types, seminoma and nonseminoma, both derived from a pre-invasive germ cell neoplasia *in situ* (GCNIS), which originates from transformed foetal gonocytes. In severely dysgenetic gonads, a GCNIS-resembling lesion is called gonadoblastoma. GCT occur rarely in young children (infantile GCT) in whom the pathogenesis is different (no GCNIS/gonadoblastoma stage) but the histopathological features are similar to the adult GCT. The rare spermatocytic tumour of older men is derived from post-pubertal spermatogonia that clonally expand due to gain-of-function mutations in survival-promoting genes (e.g. *FGFR3*, *HRAS*), thus this tumour has a different expression profile than GCNIS-derived TGCT.

Clinically most informative immunohistochemical markers for GCT, except teratoma, are genes expressed in primordial germ cells/gonocytes and embryonic pluripotency-related factors, such as placental-like alkaline phosphatase (PLAP), OCT4 (*POU5F1*), NANOG, AP-2 γ (*TFAP2C*) and LIN28, which are not expressed in normal adult germ cells. Some of these markers can also be used for immunocytochemistry to detect GCNIS or incipient tumours in semen samples.

Gene expression in GCT is regulated in part by DNA and histone modifications, and the epigenetic profile of these tumours is characterised by genome-wide demethylation, except nonseminomas. In addition, a recently discovered mechanism of post-genomic gene expression regulation involves small non-coding RNAs, predominantly micro-RNA (miR). Testicular GCT display micro-RNA profiles similar to embryonic stem cells. Targeted miRNA-based blood tests for miR-371-3 and miR-367 clusters are currently under development and hold a great promise for the future. In some patients miR-based tests may be even more sensitive than the classical serum tumour markers, β -chorio-gonadotrophin (β -hCG), α -fetoprotein (AFP) and lactate dehydrogenase (LDH), which are currently used in the clinic.

In summary, research advances have provided clinicians with a panel of molecular markers, which allow specific diagnosis of various subtypes of GCT and are very useful for early detection at the precursor stage and for monitoring of patients during the follow-up. (*Folia Histochemica et Cytobiologica* 2015, Vol. 53, No. 3, 177–188)

Key words: testis; germ cell neoplasia; testicular cancer; seminoma; embryonal carcinoma; carcinoma *in situ*; testis; spermatocytic tumour; tumour marker; PLAP; AP-2 γ ; micro-RNA; immunohistochemistry

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Abbreviations: AFP — α -fetoprotein, CIS — carcinoma *in situ* testis (currently GCNIS), DSD — disorders of sex development, EC — embryonal carcinoma, GCNIS — germ cell neoplasia *in situ* (previously CIS), GCT — germ cell tumour, β -hCG — β -chorio-gonadotrophin, LDH — lactate dehydrogenase, PGC — primordial germ cell, TDS — testicular dysgenesis syndrome, TGCT — testicular germ cell tumour

Introduction

Germ cell tumours (GCT) are a group of neoplasms with some unusual features in comparison to solid cancers that are derived from somatic cells. A striking feature of GCT is their phenotypic heterogeneity, due to retained embryonic pluripotency and subsequent aberrant somatic differentiation in a subset of these tumours, known as teratomas. This morphological heterogeneity has been causing difficulties in classification and histopathological diagnosis. During the recent decades, thanks to new tools of molecular biology, GCT have been characterised in greater detail and a number of novel diagnostic markers have been developed. In this article, we summarise research developments concerning histogenesis of different types of GCT and review immunohistochemical and serum markers which are clinically useful for differential diagnosis of GCT and their precursors.

Germ cell tumours can occur in any age, both in men and women. The tumours arise most often in the gonads but can be found in extragonadal locations, usually close to the midline of the body [1]. Malignant GCT are much more frequent in males, which can be explained by differences in biology of male and female germ cells, especially during development [2, 3]. In males, germ cell-derived malignancies occur predominantly in the testis, thus testicular GCT (TGCT) are the focus of this review.

Histopathology and pathogenesis of germ cell neoplasms

Germ cell tumours in males occur in three age groups, and in each age group the tumours are derived from a different stage of germ cell development, as illustrated in Figure 1.

The most common by far are TGCT that affect post-pubertal adolescents and young adults. Testicular tumours in this age group are derived from a pre-invasive stage, germ cell neoplasia *in situ* (GCNIS); a new term proposed by the most recent edition of World Health Organisation Classification of Tumours of the Male Genital Organs, which will be published in 2016. Previously used names were testicular carcinoma *in situ* (CIS) [4], intratubular germ cell neoplasia, unclassified (IGCNU) and testicular intraepithelial neoplasia (TIN). In severely dysgenetic gonads of individuals with disorders of sex development (DSD), the precursor lesion most often seen is gonadoblastoma. Depending on the degree of masculinisation of the gonad, either GCNIS or gonadoblastoma, or a mixture of both, may be present. GCNIS is enclosed in better masculinised tubules,

while gonadoblastoma resides in nests of granulosa-resembling cells, but the neoplastic germ cells have essentially the same phenotypic features [5–7]. A good marker for more masculinised Sertoli cells is SOX9, whereas granulosa-like cells surrounding gonadoblastoma cells express FOXL2 [8].

It is important for the understanding of the pathogenesis of GCNIS-derived TGCT that even though most of the patients are normally virilised males, these neoplasms are associated with disturbed early development of the testis, often manifested in adulthood as clusters of poorly formed tubules, undifferentiated Sertoli cells, microlithiasis and Leydig cells micronodules [9]. Among the risk factors for GCNIS/TGCT, cryptorchidism, genital malformations, low percentage sex chromosome aneuploidy and some forms of infertility, are also linked to disturbed foetal development, hence all these conditions have been grouped within testicular dysgenesis syndrome (TDS) [10]. The incidence of TGCT and some other TDS disorders have been increasing around the world, suggesting a strong environmental component in the pathogenesis, but combined with genetic susceptibility [10–12]. Discussion on the aetiology of TGCT and TDS exceeds the scope of this review, so the readers are referred to recent comprehensive review articles on this topic [11, 13, 14].

TGCT of young adults are divided into two histological entities: seminoma and nonseminoma, but a combination of the above subtypes in one patient is quite common [15]. Seminoma is the most frequent type of these tumours (approximately 60% of cases) and consists of malignant germ cells that retain a germ cell phenotype similar to GCNIS/gonadoblastoma cells. Nonseminomas display a very heterogeneous histology and are usually composed of mixtures of embryonal carcinoma, mature or immature teratoma, choriocarcinoma and yolk sac tumour (also known as endodermal sinus tumour), although in rare cases these components may occur in a pure form [15].

Embryonal carcinoma is considered a reprogrammed and transformed equivalent of embryonic stem cells and is characterised by pluripotency, which explains the presence of somatic cells within teratomatous components, which are at differentiation stages that mimic embryonic development [16]. Although this review focusses on male GCT, it is important to mention that essentially the same histological entities are recognised in females where they occur mainly in the ovary but may also arise in extragonadal locations. The ovarian and extragonadal counterparts of seminoma are dysgerminoma (may be accompanied by gonadoblastoma in females with some Y-chromosome material in their genome) and germinoma, respective-

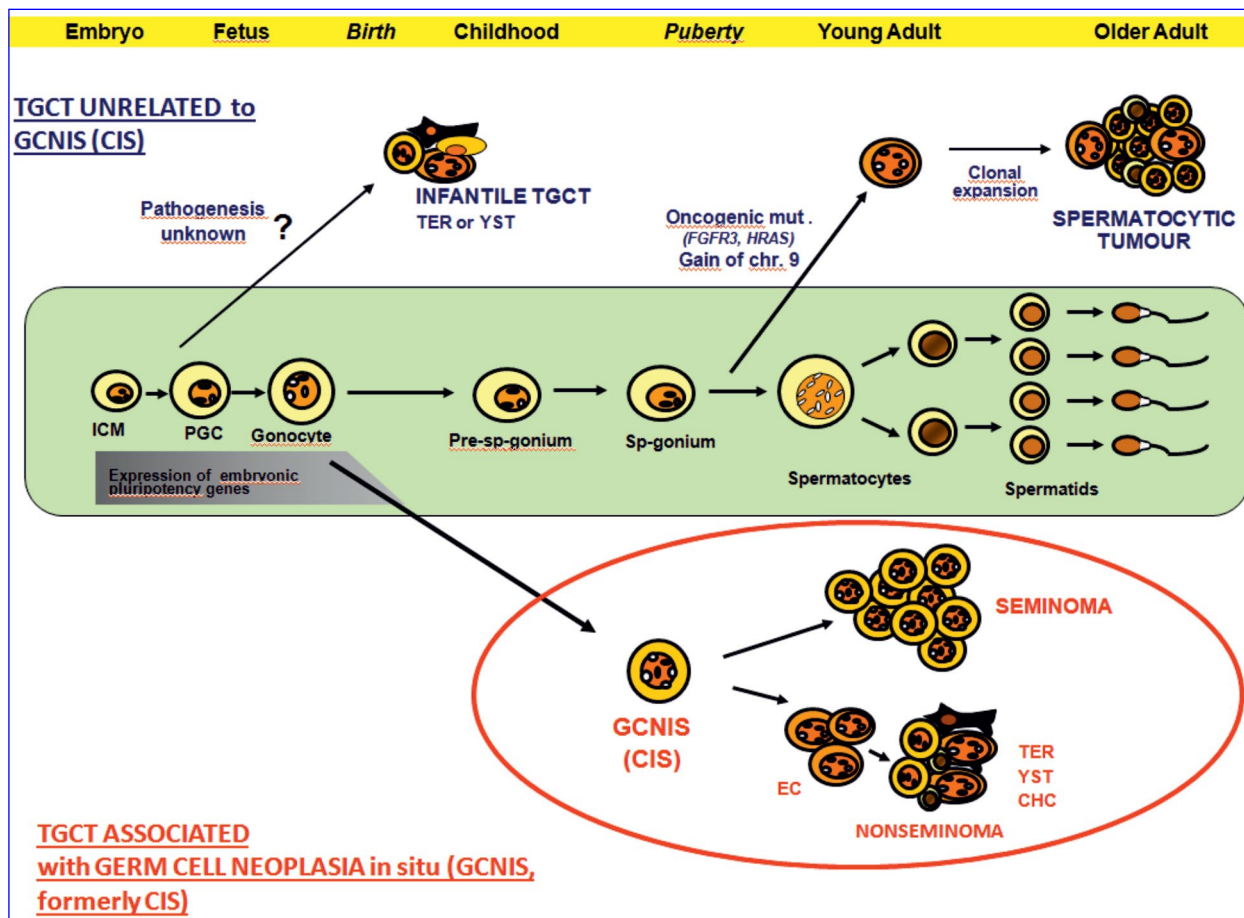


Figure 1. Schematic histogenesis of testicular germ cell tumours (TGCT) in relation to the normal development of germ cells. Normal germ cell development is shown in the green-shaded middle part of the Figure. Rare TGCT that are unrelated to germ cell neoplasia *in situ* (GCNIS); infantile TGCT and spermatocytic tumour are shown in the upper part of the graph. The most common TGCT that are derived from foetal germ cells (gonocytes) *via* the stage of GCNIS are shown in the lower part of the Figure within the red ellipse. Abbreviations: CHC — choriocarcinoma; EC — embryonal carcinoma; ICM — inner cell mass; PGC — primordial germ cell; TER — teratoma; YST — yolk sac tumour

ly, while the equivalent of nonseminoma in a female is nondysgerminoma, or simply malignant teratoma, if no other component is present [3].

Regardless of the gender, location and pathogenesis, all the above mentioned GCT have similar molecular features. The GCT genome is characterised by polyploidisation and the presence of genomic gain of the short arm of chromosome 12p, often in the form of an isochromosome, i(12p) and a relative excess of the X chromosome (reviewed in [1, 3, 17]. The presence of i(12p) is considered pathognomonic for the germ cell origin of a tumour and is useful in differential diagnosis [18, 19].

TGCT in other age groups are rare. Childhood TGCT occur typically in infants and toddlers up to 5 years of age, and histology at this age is either mature teratoma or yolk sac tumour [1, 20, 21]. Importantly, there is no GCNIS- or gonadoblastoma-like precursor

lesion, suggesting that these tumours originate directly from PGC; however, the pathogenesis remains unknown [22, 23]. These tumours, mainly mature teratomas, may also occasionally be found in adult men beyond childhood, and can be distinguished from malignant TGCT of young adults by the absence of GCNIS and lack of gain of 12p [24, 25].

The third type of TGCT, which occurs in older men, with mean age of diagnosis around 50–55 years of age is spermatocytic tumour, previously known as spermatocytic seminoma [1, 26–28]. This tumour arises exclusively in the testis (no ovarian counterpart) and is not associated with GCNIS [29]. Spermatocytic tumour originates from post-pubertal spermatogonia which expand clonally due to gain-of-function mutations in genes involved in pathways that increase spermatogonial survival/proliferation, *e.g.* *FGFR3* and *HRAS* [30]. Spermatocytic tumour is characterised

by frequent amplification of a locus on the p arm of chromosome 9, with *DMRT1* as a candidate gene [27].

Immunohistochemical markers useful for GCT diagnosis in tissue specimens

The first histochemical marker for GCNIS and GCNIS-derived TGCT was placental-like alkaline phosphatase (PLAP), previously widely used in animal models to detect PGCs [31]. The long postulated by our group similarity of GCNIS cells to foetal gonocytes [32] was first supported by immunohistochemical studies which looked at one or few proteins at the time [33–36]. Subsequently, a genome-wide microarray study corroborated this hypothesis by a direct comparison of GCNIS and gonocyte transcriptomes [37].

Likewise, pluripotent characteristics of GCT observed previously by pathologists, has been demonstrated by molecular studies, including numerous microarray studies, which detected in these tumours (excluding teratoma) as well as in human PGCs/gonocytes, a high expression of embryonic factors involved in pluripotency regulation, such as *POU5F1*(OCT4), *NANOG*, *SOX2*, *REX1*, *UTF1* or *LIN28* [38–47]. These studies have also identified differences between the GCT subtypes, e.g. seminoma/(dys)germinoma versus embryonal carcinoma or yolk sac tumour. It is not possible to list here all studies and all implicated genes, so the interested reader should consult recent systematic review articles [3, 48].

The above-mentioned studies provided a range of markers that have been validated for the identification of GCNIS and seminoma/(dys)germinoma in tissue specimens by immunohistochemistry, including OCT4 [36, 39, 49, 50], *NANOG* [51, 52], *LIN28* [47, 53], *AP-2 γ* [54–56], podoplanin (PDPN, M2A, D2-40, Aggrus) [57, 58], and *KIT* [33]. The markers that are useful in clinical practice and research are listed in Table 1. Immunohistochemistry is very helpful for the detection of GCNIS in testicular biopsies [59–61] and we suggest using at least two different markers for each tissue specimen, e.g. PLAP and OCT4 or PDPN (Figure 2).

For differential diagnosis of nonseminomas, a panel of several markers has to be used in order to identify the presence of different histological components, as shown in Table 1. For the detection of embryonal carcinoma, pluripotency markers OCT4 and *NANOG* are useful, but additional discriminative markers are needed to distinguish it from seminoma. We suggest to use *SOX2* [43, 44, 62, 63] (Figure 3) or *CD30* (both are positive in embryonal carcinoma but negative in seminoma), simultaneously with *KIT* or PDPN (positive in seminoma but negative in embryonal

carcinoma) [64, 65]. Differentiated somatic elements within teratomas have expression profiles of various tissue lineages and no longer express pluripotency factors or germ cell-specific genes.

Extraembryonic components yolk sac tumour and choriocarcinoma are usually admixed with other nonseminomatous elements, but may occur as pure tumours, especially in children. Regardless of the age and gender of the patient, these components have essentially the same morphology and expression profiles [3, 21, 43, 48, 65]. Yolk sac tumour produces and secretes α -fetoprotein (AFP), which is detectable in serum and tumour tissue [66]. Among other immunohistochemical markers for yolk sac tumour the most informative are Glypican-3 (GPC3) and *SALL4* [65, 67, 68] (Figure 3). Choriocarcinoma contains trophoblastic-like syncytia and secretes β -chorio-gonadotrophin (β -hCG) which is measurable in serum and detectable in tissues [66]. Other immunohistochemical markers, such as GPC3 or *SALL4* are present but are not specific for choriocarcinoma [69].

As far as spermatocytic tumour of older men is concerned, its gene expression profile — both at the transcript and protein level — resembles closely profiles of spermatogonia and early primary spermatocytes of the adult testis [26, 27, 70]. Accordingly, spermatocytic tumours do not express embryonic pluripotency genes and PGC/gonocyte specific markers, and markers of haploid spermatids (e.g. p19-INK4) are also negative [26]. Among the best markers for spermatocytic tumour are the so-called cancer-testis antigens: *SSX1-4*, *MAGE-A4*, *MAGE3*, *NY-ESO-1/CTAG1A*, *GAGE4*, *SAGE1* [27, 70–74] (Figure 3). Other immunohistochemical markers include *SYCP1*, *NSE* (neuron-specific enolase), *CHK2*, *VASA*, *UTF1*, *FGFR3* or *HRAS*, and *DMRT1* [26, 27, 30, 46, 71]. Recently, *OCT2* was detected in a small subset of spermatocytic tumours and in A-dark spermatogonia [70], and in both cell types, *OCT2* was found mutually exclusive with *DMRT1* [75]. For diagnostic purposes in the clinics we suggest *MAGE-A4* (Figure 3) and *DMRT1*, which are the most robust immunohistochemical markers, as shown in Table 1.

Detection of GCNIS in semen samples by immunocytochemistry and histochemistry

Like other precancerous *in situ* changes, GCNIS can be detected before the invasive tumour has developed. This happens only rarely, mainly in patients who undergo testicular biopsies because of an increased risk of TGCT, such as DSD, contralateral testicular cancer, history of cryptorchidism or infertility, in particular if the testes are small and microlithiasis is

Table 1. Immunohistochemical markers of germ cell tumours (GCT). The markers are grouped according to their expression in normal germ cells during development; primordial germ cell (PGC) and gonocyte markers are listed first, markers specific for more mature germ cells are listed next, and proteins not expressed in germ cells are listed at the bottom of the table. The most informative markers for each cell/GCT type are marked with grey background. Expression: '+' — positive, '+/-' or '-/+' — heterogenous, '-' — negative

Protein/antigen (alternative names)	Normal germ cells				GCNIS/GDB	Seminoma/(Dys)Germinoma	Nons seminoma components				Spermatocytic tumour	Comments
	PGC Gonocytes	Spermatogonia	Spermatocytes	Spermatids			EC	YST	CHC	Teratoma		
OCT4 (POU5F1)	+	-	-	-	+	+	+	-	-	-	-	Pluripotency factor
NANOG	+	-	-	-	+	+	+	-	-	-	-	Pluripotency factor
LIN28	+	-	-	-	+	+	+	-/+	-	-	-	
AP-2γ (TFAP2C)	+	-	-	-	+	+	+	-/+	-	-	-	Required for germ cell specification
PLAP	+	-	-	-	+	+	+	-/+	-	-	-	Some antibodies cross-react with myoid cells
PDPN (M2A, D2-40, Aggrus)	+	-	-	-	+	+	+	-/+	-	-	-	Also present in immature Sertoli cells and in lymphatic vessels
KIT (CD117)	+	-/+	-	-	+	+	+	-/+	-	-	-/+	Present in many extra-gonadal tissue stem cell types (e.g. melanocytes)
SALL4	+/-	+	-	-	+	+	+	+	+/-	-	+/-	
DMRT1	+/-	+/-	-	-	+	-/+	-	-	-	-	+	Not expressed in A-dark spermatogonia
MAGE-A4	-	+	+/-	-	+	-/+	-/+	-/+	-	-	+	Expressed in some somatic tumours (e.g. melanoma)
CD30	-	-	-	-	-	-	+	+	-	-	-	
SOX2	-	-	-	-	-	-	+	+	-	-	-/+	
Glypican 3 (GPC3)	-	-	-	-	-	-	-	-	+	+	+/-	
AFP	-	-	-	-	-	-	-/+	+	+	+	-/+	Present in liver carcinomas
β-hCG	-	-	-	-	-	-	-	-	-	+	-	Placental trophoblast

Tumour name abbreviations: GCNIS — germ cell neoplasia *in situ*; GDB — gonadoblastoma; EC — embryonal carcinoma; YST — yolk sac tumour; CHC — choriocarcinoma

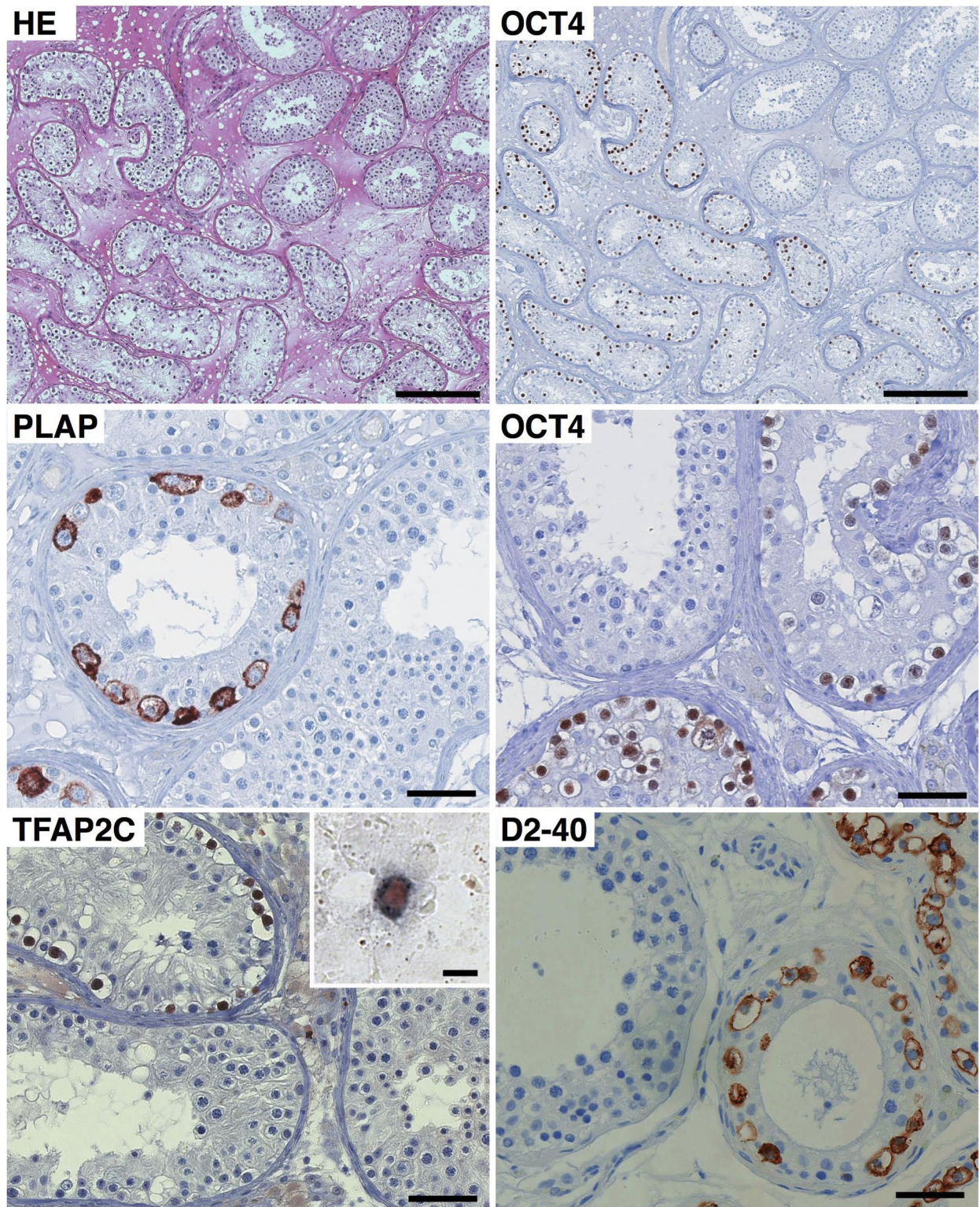


Figure 2. Germ cell neoplasia *in situ* (GCNIS): histology and selected immunohistochemical markers. The two top microphotographs are haematoxylin-eosin (HE)-stained (left) and OCT4-stained (right), serial sections of an adult testis specimen with GCNIS (visible on the left of both images) and tubules with normal spermatogenesis (on the right); scale bar = 250 μ m. The staining for OCT4 clearly helps to detect GCNIS. Sections stained with four commonly used immunohistochemical markers (PLAP, OCT4, TFAP2C and D2-40) are shown at higher magnification below; scale bar = 50 μ m. Tubules with GCNIS are shown side-by-side to tubules with ongoing spermatogenesis. Note an insert in the micrograph with TFAP2C (also known as AP-2 γ), showing a GCNIS cell in a semen smear positive for TFAP2C (red colour, in the nucleus) and displaying a strong enzymatic reaction of alkaline phosphatase (blue colour, in the cytoplasm); scale bar = 10 μ m

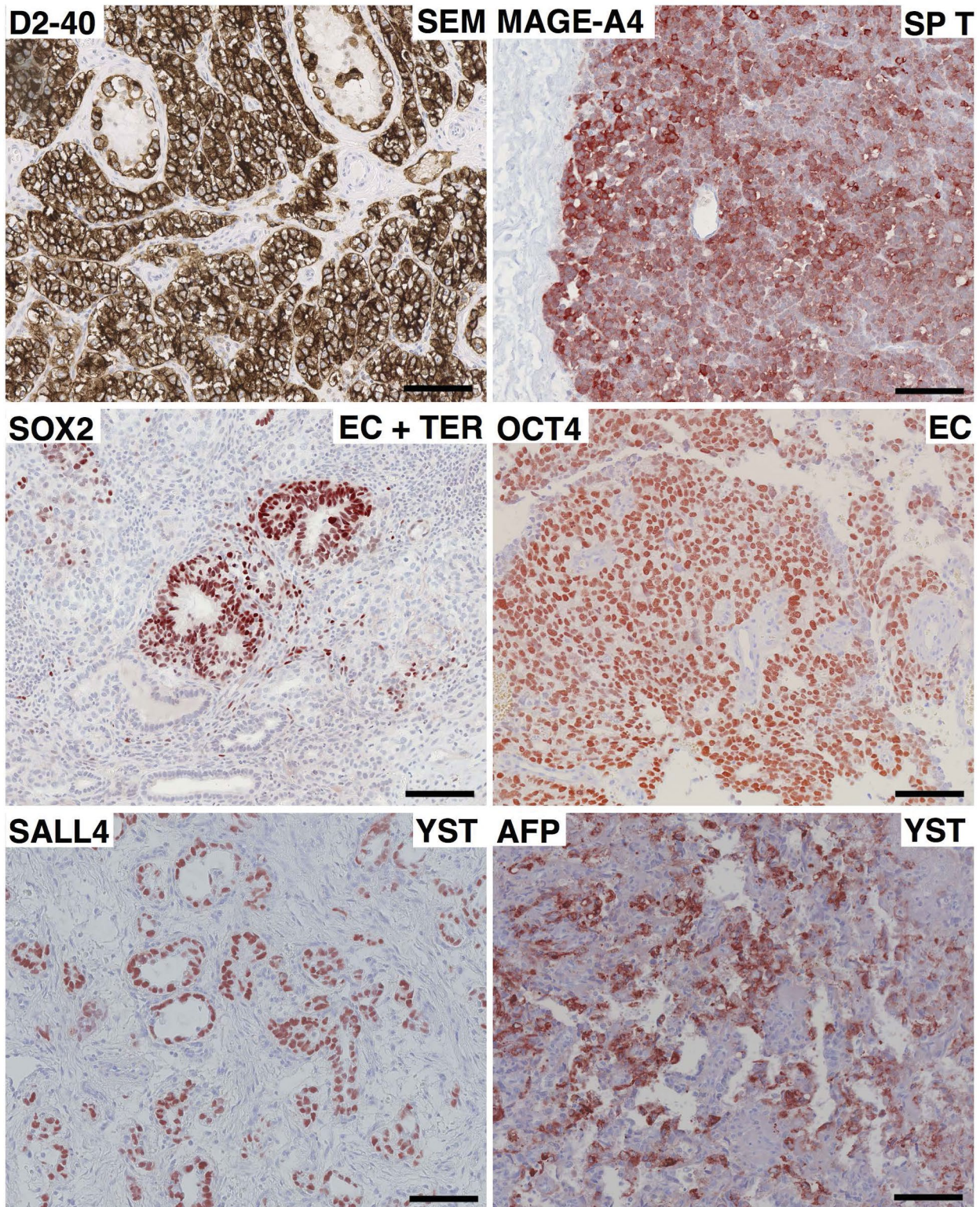


Figure 3. Examples of immunohistochemical markers for different histological subtypes of testicular germ cell tumours. From the top: D2-40 in a seminoma (SEM), there are also two tubules with germ cell neoplasia *in situ* (GCNIS) visible; MAGE-A4 in a spermatocytic tumour (SPT), SOX2 and OCT4 in embryonal carcinoma (EC) containing differentiated teratomatous tissues (TER), the latter component negative for both markers, SALL4 and AFP in two different specimens of yolk sac tumours (YST). Scale bar = 100 μ m

visible on ultrasound examination [59, 60, 76, 77]. Giwercman was the first to demonstrate that GCNIS cells are occasionally exfoliated into semen and can be identified in the ejaculate by immunostaining for M2A/PDPN antigen [78]. However, cell surface markers were not optimal for this purpose, so more recently, we and others have exploited nuclear proteins, AP-2 γ or OCT4, which ought to be better protected from degradation in semen [79, 80]. The test was promising but had rather low sensitivity and required good experience to distinguish cells which were truly positive from occasionally false positive cells [79]. In another study from France, cancer-testis antigens, MAGE-A4 and NY-ESO-1 have been tested for suitability for detecting GCNIS cells in semen [81]. However, a serious drawback of this approach is that both antigens are also expressed in normal germ cells (spermatogonia and early primary spermatocytes), thus leading to false-positive results in healthy subjects. Subsequently, our group has improved sensitivity of the AP-2 γ -based test by combining it with rapid histochemical staining detecting enzymatic activity of PLAP [82]. The double-staining method was further optimised by automated staining, slide scanning and imaging software, which increased the speed and objectivity of the test [83]. An example of GCNIS cells detected by this method is shown as an insert in Figure 1. This approach is reliable in experienced hands, but the sensitivity of the assay remains low, because GCNIS cells in many patients do not readily exfoliate into semen. It follows that a negative result does not completely exclude the presence of a neoplasm in a testicle. However, the test is much less invasive than a surgical biopsy and can be performed repeatedly, so it may be considered to monitor for GCNIS also those patients who do not have a stringent indication for testicular biopsy.

Immunohistochemical markers of epigenetic modifications in TGCT

In addition to the embryonic gene expression pattern, epigenetic profiles of neoplastic germ cells resemble those of primordial germ cells (PGCs) and foetal gonocytes to a large extent [84, 85]. DNA methylation and histone modifications in GCNIS and TGCT are different from somatic cells, but also strikingly variable. Consistent DNA demethylation throughout the genome is observed in GCNIS and seminoma, whereas the genome of non-seminomas is methylated in a non-random manner with imprinted genes left hypo-methylated [85–88]. This can be easily demonstrated in tissue specimens by immunohistochemical staining for 5-methyl-cytosine [84, 87].

The hypomethylation of GCNIS is apparently actively maintained by expression of APOBEC1 and base excision repair proteins MBD4, APEX1 and PARP1 [89]. A very different picture is displayed by spermatocytic tumour which originates from clonally-expanding mature spermatogonia, where hypo- and hyper-methylated cells can be seen admixed next to each other, in a seemingly chaotic pattern [90].

In addition to DNA methylation, modifications of histones in nuclear chromatin are another mechanism of gene expression regulation. Histone modifications are variable in TGCT and can also be detected by immunohistochemistry. GCNIS cells are characterised by the virtual absence of restrictive histone modifications H3K9me2/3 and H3K27me3, and the abundance of H3K4me1, H3K4me2/3, H3K9ac and the histone variant H2A.Z, which are all modifications associated with active and permissive chromatin structure [84, 91]. Moreover, GCNIS cells show high levels of H4/H2AR3me2 and simultaneously express BLIMP1/PRMT5, which is essential for PGC specification [92]. We hypothesise that the DNA hypo-methylation, the absence of DNA damage response and a high proliferation rate combined with ‘permissive’ chromatin modifications may render GCNIS vulnerable to genomic instability and increased plasticity in response to exogenous factors [93].

Traces of GCT in blood: classical biochemical markers and micro-RNAs

Diagnosis of GCT in the clinic involves an obligatory step of taking a blood sample to measure by immunoassays a set of biochemical tumour markers; β -hCG, AFP and (in some centres) lactate dehydrogenase (LDH, LD-1) [94, 95]. These markers are helpful in the diagnosis and monitoring of GCT and are included in commonly used staging manuals. AFP and β -hCG are secreted by nonseminomatous tumours, yolk sac tumour and syncytiotrophoblast of choriocarcinoma (as mentioned above in the section on immunohistochemical markers), whereas LDH is also secreted by seminoma. The presence of just a few giant cells in a seminoma may be sufficient to detect β -hCG in serum. LDH levels in serum may be increased if the tumour has a prominent gain of chromosome 12p, where the *LDHB* gene is located [96]. The interpretation of serum levels of these markers in patients with seminoma, pure EC and teratoma is sometimes difficult, and many cases are marker-negative.

However, it can be expected that a new blood tests will become soon available. A recently discovered mechanism of post-genomic gene expression regulation involves small non-coding RNAs, predominantly

micro-RNA (miRNA or miR), which are among the smallest (~22 bases long) RNAs, thus much less prone to degradation. MiRNAs inhibit gene expression by direct base-pairing with transcripts, and one miRNA may have many targets. These small RNAs act inside the cells, but recently it has become clear that miRNAs are actively secreted in exosome vesicles that can affect post-transcriptional regulation in a remote recipient cell. GCT display micro-RNA profiles similar to embryonic stem cells, and these embryonic miRNA types are already highly upregulated and detectable in pre-invasive GCNIS cells [97]. Interestingly, the characteristic miRNA profiles are similar in GCT that arise in children and adults, and are detectable in all histological subtypes and components, except teratoma [21, 98, 99]. This specific TGCT profile, which was detected by several groups independently from each other, includes a high level of miR-371-3 cluster, miR-302 and miR-367. Tests targeting these specific miRNAs are currently under development. Preliminary studies have already proven great specificity (99–100%) and sensitivity of these tests for the detection of TGCT [100–102]. Other body fluids are also suitable for detection of miRNA released by disseminated tumours, e.g. pleural effusion fluid. Importantly for testicular neoplasms, seminal plasma apparently also has elevated levels of specific miRNAs compared with controls [103]. These results are very promising and it is likely that miRNA-based assays will be routinely used for diagnosis and follow-up of the patients in not so distant future.

Conclusions

In conclusion, research advances over the years have provided clinicians with a panel of molecular markers, which allow specific diagnosis of germ cell tumours in a clinical laboratory setting. Immunohistochemical and histochemical markers in tissues remain used and are still the gold standard for pathology diagnosis. However, the existing biochemical serum assays will soon be supported — and in the future perhaps replaced — by novel more sensitive and specific tests that detect circulating products of tumour cells and are likely to improve early detection and monitoring of patients.

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