Age-related biological features of germ cell tumours

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Abstract

Germ cell tumours (GCTs) are rare but clinically and pathologically diverse tumours that occur in an extensive range of age groups, from children to older adults and which include both seminomatous and nonseminomatous tumours. Current clinical management for both male and female teenagers and young adults (TYAs) with GCTs remains inconsistent, alternating between paediatric and adult multidisciplinary oncology teams, based on locally defined age cut-offs. We therefore reviewed available literature to determine the biological similarities and differences between GCTs in young children [0-12 years (y)], TYAs (13-24y), and older adults (>24y). GCTs arising in paediatric and adult populations in general showed marked molecular biological differences within identical histological subtypes, whereas there was a distinct paucity of available data for GCTs in the TYA population. These findings highlight that clinical management based simply on chronological age may be inappropriate for TYA and suggests that the optimal future management of GCTs should consider specific molecular biological factors in addition to clinical parameters in the context of patient specific age group specialty.

Aims

The aim of this review was to analyse molecular biological studies of germ cell tumours (GCTs) arising in the paediatric (≤12y) and older adult (>24y) age groups for key similarities and differences in an attempt to identify where the biology of GCTs from teenagers and young adult (TYA) patients lies within this framework. The review discusses current knowledge in this area and also highlights gaps that still need to be addressed. In the UK, the TYA age group includes male and females aged 13-24y; accordingly we have used this definition for the purposes of this review). The aims were achieved through a synthesis and analysis of available biological data for GCTs in children, TYAs and older adults. We anticipated that the goal would be to use a combination of both clinical and biological risk factors to improve clinical management decisions for TYA patients with GCTs (Murray et al., 2009).

Background

Regardless of patient age, tumour site and histological subtype, all GCTs are believed to originate from primordial germ cells (PGCs) (Teilum, 1965). The histological appearance of these tumours varies depending on the degree of germ cell differentiation when carcinogenesis takes place (Ross et al., 1999). GCTs can be separated into two major histological subgroups, seminomatous and nonseminomatous tumours. Seminomas (or germinomas, collectively referring to testicular seminomas, ovarian dysgerminomas and extragonadal germinomas) are composed of uniform sheets of tumour cells that resemble undifferentiated PGCs. By contrast, nonseminomas exhibit varying degrees of differentiation that resemble particular cell lineages or stages of embryonic development and include teratomas, embryonal carcinomas (ECs), yolk sac tumours (YSTs) and choriocarcinomas (CHCs) (Palmer et al., 2008). In addition to these, mixed malignant GCTs also exist which are composed of more than one histological subtype.

GCTs are rare, accounting for 4% of all childhood malignancies (Surti et al., 1990). Within this age range, they exhibit a characteristic bimodal age distribution, with a relatively high incidence in the first few years of life, which then declines to very low levels before increasing again in adolescence. As a result, their incidence in the paediatric (4 per million) and older adult (14 per million) populations remains markedly lower than in TYAs (60 per million in males and 8 per million in females) (Figure 1) (Murray et al., 2009).

GCTs can arise as a painless mass in both gonadal and extragonadal sites, including the sacrococcygeum, vagina, mediastinum and central nervous system, typically in the pituitary and pineal region in the case of the latter. It has been proposed that the location of these midline sites may be accounted for by an aberrant path taken by PGCs during their migration from the yolk sac to the gonadal ridge during embryogenesis (Looijenga and Oosterhuis, 1999; Gilbert et al., 2011). In childhood, only 50% of GCTs are gonadal, but this figure increases to 95% in adulthood (Nicholson and Palmer, 2010); where TYA GCTs lie within this spectrum remains unclear.

Historically, malignant GCTs (MGCTs) were rapidly fatal (Nicholson and Palmer, 2010). However, with the advent of effective platinum-based chemotherapy, MGCTs became highly curable, even when diagnosed at advanced clinical stages. The use of bleomycin, etoposide and cisplatin (BEP) in adults with advanced stage GCTs now results in overall survival rates in excess of 90% (Horwich et al., 2006; Krege et al., 2008). Although paediatric GCTs were initially treated with an adult cisplatin-based regime, the neurological and ototoxic sequelae of treatment in young children had a substantial impact upon quality of life, driving the UK to adopt a carboplatin, etoposide and bleomycin (JEB) based approach instead (Mann et al., 1989; Hale et al., 1999). In children, JEB showed comparable survival for all tumour stages (Mann et al., 2000; Stern and Bunin, 2002). Recently, a joint Malignant Germ Cell International Collaboration (‘MaGIC’) initiative, established between the USA and UK, pooled data from more than 1,100 paediatric patients with GCTs who

had received platinum-based chemotherapy (Hale et al., 2012). The prognostic features of tumours in children and TYAs with GCTs were determined, comparing the outcomes from the US cisplatin-based (PEB) and the UK high dose carboplatin-based approaches (JEB). The results showed that after adjustment for other prognostic factors, the risks of failure for JEB and PEB were not significantly different for patients <11y of age (Hale et al., 2012). In adults, carboplatin therapy has been shown to be inferior to cisplatin, although lower dose carboplatin regimes are typically used in this age group due to the increased risk of myelosuppression (Bokemeyer et al., 1996; Horwich et al., 1997). In younger children, however, higher dose carboplatin resulted in survival rates equivalent to those achieved with cisplatin. These differences may be due to a more aggressive tumour phenotype in older patients, reflect the poor deliverability and tolerance of higher dosing schedules in adults, or a combination of these two factors. It therefore remains unclear as to whether the optimal treatment for TYA GCTs should be as for paediatric cases with relatively high dose carboplatin, or as for adult tumours with cisplatin. If the differing responses to carboplatin therapy are due to tumour biology rather than to treatment dose, then treating TYAs with higher dose carboplatin is likely to be ineffective. In order to know if higher carboplatin doses should be trialled in TYAs, it is necessary to determine whether their GCT biology is most akin to that of paediatric or adult GCTs. This calls for TYA patient-focused clinical studies aimed at establishing their carboplatin dose tolerance, as has been described for acute lymphoblastic leukaemia (Boissel et al., 2003). Ultimately, this may allow clinicians to combine excellent overall survival rates with the potential for reducing the known late neurotoxicity, nephrotoxicity and cardiovascular toxicity associated with cisplatin use. There is ongoing discussion between paediatric and adult (testicular and ovarian) GCT specialists and trialists to consider the application of this in a prospective trial.

The relationship between age, epidemiology and histology in GCTs has been highlighted by Oosterhuis and Looijenga in the design of their classification system (Oosterhuis and Looijenga, 2005). This system divides GCTs into five distinct types. Type I tumours occur in neonates and

infants <5y of age and predominantly comprise nonseminomatous teratomas and YSTs. Type II tumours include both nonseminomatous and seminomatous GCTs and affect the TYA population (Oosterhuis and Looijenga, 2005). As Type III tumours (which comprise spermatocytic seminomas occurring in the elderly male population) are not thought to arise from PGCs (Looijenga et al., 2007), they will not be considered further in this review. Finally, benign ovarian teratomas are categorised as Type IV tumours, while Type V tumours consist of hydatidiform moles (a benign form of gestational trophoblastic disease). According to this classification, GCTs arising in the TYA population should be labelled as Type II tumours. Although this classification is simple, it remains unclear how widely applicable it is to the broad spectrum of paediatric and adult GCTs observed in clinical practice, and whether it can be applied to the TYA GCT population. This review of biological GCT studies in paediatric and adult patients therefore attempts to discern whether a TYA tumour is likely to represent a mature onset childhood-type tumour (Type I), the early onset of a tumour more typical of an older adult (Type II), or in fact represents a distinct group. Importantly, the specific age at which GCTs should be managed as childhood or adult disease remains undefined, resulting in the inconsistent application of treatment regimes and likely poorer outcome for TYAs with GCTs (Murray et al., 2009). We propose that specific features in the biology of these tumours may shed light upon the transition from paediatric to TYA GCTs, and from TYA to adult GCTs, thus enabling rational clinical management decisions to be made for these age groups.

GCT biology

Recent years have witnessed improvements in our overall biological understanding of MGCTs. The similarities and differences in the biology of GCTs within the paediatric and adult age range are discussed, with particular reference to pre-invasive disease, genomic imprinting, chromosomal aberrations, susceptibility loci and both protein-coding and non-protein-coding gene (microRNA) expression.

Pre-invasive disease

All adult testicular GCTs (TGCTs) are thought to arise from a common precursor lesion, originally termed ‘carcinoma *in situ*’ (Skakkebaek, 1972). The currently accepted terminology is ‘intratubular germ cell neoplasia unclassified (IGCNU)’ due to the absence of epithelial differentiation within these lesions. IGCNU appears to develop from undifferentiated PGCs based on studies demonstrating their similar morphology, imprinting patterns and gene expression (van Gurp et al., 1994; Almstrup et al., 2004; Rajpert-De Meyts, 2006). IGCNU does not spontaneously regress and eventually progresses to GCT (Oosterhuis and Looijenga, 2005) in approximately 50% of patients within 5 years (Skakkebaek, 1978; Maase et al., 1986; Giwercman and Skakkebaek, 1993). This progression is highlighted by the fact that IGCNU and TGCT occur at similar frequencies and that IGCNU is found in testicular tissue adjacent to GCTs in 90% of adults (Jacobsen et al., 1981; Giwercman et al., 1991; Dieckmann and Skakkebaek, 1999; Huyghe et al., 2005). This is in marked contrast to paediatric GCTs, where multiple studies have shown IGCNU to be absent (Manivel et al., 1988; Manivel et al., 1989; Jorgensen et al., 1995; Hawkins et al., 1997; Fan and Ulbright, 2012). Indeed, IGCNU in paediatric GCTs has only been described in a small number of isolated cases (Hu et al., 1992; Stamp et al., 1993; Renedo and Trainer, 1994). However, others have questioned whether these paediatric cases truly represent IGCNU (Hawkins et al., 1997). These observations suggest that the presence or absence of IGCNU is likely to prove clinically useful in distinguishing adult from paediatric TGCTs, and, furthermore, that TGCTs in infants and children are likely to arise through a biologically distinct mechanism to TGCTs in adulthood (Hawkins et al., 1997). By contrast, the implications for TYA TGCTs remain unclear given that the exact frequency of IGCNU is unknown in this age group.

Genomic imprinting

Imprinting is an epigenetic phenomenon by which certain genes are selectively differentially expressed in a parental allele-specific manner such that 'silenced' (imprinted) genes are not expressed. This mechanism forms an integral part of the regulation of embryonic cell development (including both in the fetus and placenta). Regulated expression of the imprinted genes *H19* and *IGF-2* (*insulin-like growth factor-2*) is necessary for normal human embryo development (van Gurp et al., 1994). As GCTs are thought to originate from early germ cells, the patterns of allelic expression of the *H19* and *IGF-2* genes were obvious candidates for investigating whether aberrant genomic imprinting is involved in GCT pathogenesis. During normal development, PGCs establish a new sex-specific imprinting pattern. Assuming that GCTs preserve the imprinting status of their cell of origin, they would not be expected to exhibit any imprinting pattern if they have arisen from premeiotic germ cells prior to their arrival at the gonadal ridge (i.e. they would display biallelic expression of both *H19* and *IGF-2*). However, if GCTs develop from PGCs that have entered meiosis instead, they will display a sex-specific imprinting pattern (Schneider et al., 2001). In this regard, the pioneering work of van Gurp and colleagues in adult GCTs reported biallelic expression of *IGF-2* in 55% of TGCTs and biallelic *H19* expression in 70% of TGCTs (van Gurp et al., 1994). The consistent expression of both parental alleles of the *H19* and *IGF2* genes implies that TGCTs may develop from precursor cells that have either erased their imprint or been subjected to a relaxation of the normal monoallelic expression of these genes ensuing from demethylation at the imprinted allele (van Gurp et al., 1994). This is consistent with the finding of Ross and colleagues who also demonstrated loss of imprinting (LOI) of *H19* and *IGF-2* genes in both paediatric and TYA GCTs (Ross et al., 1999). Their results suggested that while LOI of *H19* and *IGF-2* may be common in paediatric and TYA TGCTs, there was a variable pattern of LOI in ovarian GCTs in these age groups, which was proposed to reflect the stage of imprinting during germ cell development at which tumorigenesis occurred.

Schneider and colleagues reported a multipoint imprinting analysis of the *H19*, *IGF-2* and *SNRPN* (*small nuclear ribonucleoprotein polypeptide N*) genes in 42 gonadal and extragonadal GCTs in children and TYAs (Schneider et al., 2001). Forty-six percent of informative tumours showed biallelic *H19* expression, while 47% showed biallelic *IGF-2* expression. Eighty-four percent of gonadal GCTs and 81% of extragonadal GCTs showed absence of *SNRPN* methylation consistent with LOI. Crucially, the frequency of LOI between gonadal and extragonadal GCTs was comparable, and did not correlate with patient age. These results suggest that both gonadal and extragonadal GCTs are derived from PGCs, and have consistent LOI of *SNRPN* and partial LOI of *H19* and *IGF-2*. When compared with adult TGCTs, the frequency of LOI was lower in paediatric testicular and extragonadal GCTs, and in TYA ovarian GCTs. As such, LOI of *H19* and *IGF-2* has been found in the paediatric, TYA and older adult populations although LOI patterns varied across the three populations (van Gurp et al., 1994; Nonomura et al., 1997; Ross et al., 1999; Schneider et al., 2001). Although this suggests that these tumours all arise from PGCs, this may nevertheless occur at different stages of germ cell development. In particular, the partial LOI in paediatric GCTs indicates that these tumours may likely arise from a more immature germ cell, and that TYA GCTs are more similar to GCTs in older adults (Schneider et al., 2001).

Although many chromosomal aberrations in GCTs have been established, the major tumour suppressor genes (TSGs) associated with such areas of physical or functional genomic losses remain to be identified. It has been demonstrated that in adult TGCTs, methylation of the TSGs *APC* (*adenomatous polyposis coli*), *RASSF1A* (*Ras association domain family 1 isoform A*) and *MGMT* (*O-6-methylguanine-DNA methyltransferase*) occurs more frequently in nonseminomas compared to seminomas (Honorio et al., 2002). Methylation of the TSGs *RASSF1A* and *HIC1* (*hypermethylated in cancer 1*) have been shown to be associated with treatment resistance, whilst methylation of *MGMT* is associated with platinum sensitivity (Koul et al., 2004). In paediatric GCTs *APC*, *RASSF1A* and *HIC1* have all been shown to be significantly more methylated in YSTs

than in germinomas (Jeyapalan et al., 2011). In paediatric YSTs, methylation of the TSG *RUNX3* (*Runt-related transcription factor 3*) has been reported, an imprint which has not been reported in adult YSTs, and which may therefore contribute to the pathogenesis of this childhood GCT subtype (Kato et al., 2003; Furukawa et al., 2009). In line with this, a recent study examined the methylation status of 15 selected TSGs in 28 paediatric GCT samples and found deletion of the 1p36-p35 regions in 19 (68%) cases (Ichikawa et al., 2013). *RUNX3*, located at 1p36, was methylated in 16 of the 19 (84%) tumours, demonstrating that hypermethylation appears to be the principal mechanism of inactivation of this gene in paediatric GCTs.

Chromosomal aberrations

The ploidy status of GCTs was reviewed by Veltman and colleagues, who found that TGCTs in TYA and older adult populations were usually aneuploid, with possible overall differences between histological subtypes (Veltman et al., 2003). Seminomas were found to be hypertriploid, whilst nonseminomas were hypotriploid (Looijenga, 2001). By contrast, the pattern of genomic gains and losses between the histological subtypes was broadly similar, except for gains of chromosome 15 in seminomas compared to nonseminomas (Looijenga, 2001). In the paediatric population, DNA ploidy was found to be variable (diploid, triploid and tetraploid) (Looijenga and Oosterhuis, 1999; Bussey et al., 2001; Schneider et al., 2001) while ovarian GCTs are mostly diploid, independent of age (Surti et al., 1990).

Over three decades ago, Atkin and Baker detected an isochromosome of the short arm of chromosome 12 [i(12p)] in TGCTs based on the karyotypic analysis of metaphase chromosomes (Atkin and Baker, 1982). It was subsequently established that i(12p) occurs in 80% of adult TGCTs, while gain of 12p genomic material is invariably present in the other 20% of cases (Looijenga, 2001). Indeed, i(12p) appears to be a characteristic cytogenetic event in both gonadal and extragonadal adult GCTs (Oosterhuis et al., 1997; Bussey et al., 1999; Kraggerud et al., 2000;

Schneider et al., 2002; McKenney et al., 2007; Palmer et al., 2007). By contrast, a gain of 12p was until recently thought to occur only rarely in paediatric cases. In early studies, Perlman and colleagues used comparative genomic hybridization (CGH) to screen for genetic abnormalities in 16 paediatric (<4y) YSTs (10 testicular site and six extragonadal) (Perlman et al., 2000). Gain of 12p was seen in only one of their 16 tumours (a testicular tumour), highlighting the dissimilarity in genomic aberration profile between paediatric and adult GCTs. Bussey and colleagues assessed 12p status in 18 male GCTs, five of which had a 12p gain (Bussey et al., 2001). More specifically, no 12p gain was seen in tumours from patients <9y of age. This led the authors to postulate that gain of 12p was significantly associated with patient age and, accordingly, may relate to the physiological changes brought about by the onset of puberty. By contrast, a more recent paediatric (0-16y) study (Palmer et al., 2007) analysed 11 seminomas, 22 YSTs and one metastatic EC from a range of anatomical sites from 12 male and 22 female patients, including a large proportion of ovarian GCTs. Chromosome 12p gain was identified in 44% of cases, with an incidence increasing with age (29% of those <5y, compared with 53% in those 5-16y). Again, these findings suggest that genomic copy number imbalances can distinguish GCT subgroups primarily by age, rather than by tumour site or histology.

Given that the only consistent structural chromosomal abnormality in TGCTs is 12p gain [predominantly due to i(12p)], the implication is that this genomic aberration is associated with MGCT development. In support of this theory, studies of IGCNU demonstrate a similar pattern of overall gains and losses to those observed in invasive TGCTs, except for 12p gain (Rosenberg et al., 2000; Summersgill et al., 2001; Ottesen et al., 2003).

With regard to paediatric tumours, Schneider and colleagues identified some of the key genomic changes found in mediastinal GCTs in this age range (Schneider et al., 2002). They also reported

that mediastinal teratomas exhibited no genomic gains or losses. By contrast, in young children (<8y of age) mediastinal MGCTs displayed the same pattern of gains and losses as those found in age-matched sacrococcygeal and testicular GCTs, including gain of 1q, 3, and 20q, and loss of 1p, 4q and 6q. Similarly, for children ≥8y of age, mediastinal MGCTs showed identical profiles to gonadal GCTs, particularly with respect to 12p gain. The authors stated that mediastinal MGCTs arising in TYAs carried a poorer prognosis compared to those affecting young children (<8y), likely due to biological age-dependent differences in these tumours (Schneider et al., 2002). Indeed, studies have identified differences in the clinical behaviour of tumours with the same histological subtype, suggesting that these may reflect inherent differences in tumour biology amongst different age groups (Schneider et al., 2002; Marina et al., 2006).

Cytogenetic data on ovarian MGCTs are limited, and the frequency of 12p gain in female GCTs in different age groups is not well established. In paediatric female GCTs, the presence of i(12p) is infrequent, with few, individual cases being reported (Speleman et al., 1990; Speleman et al., 1992; Bussey et al., 1999; Bussey et al., 2001). By contrast, i(12p) is more frequent in adult female GCTs, particularly in dysgerminomas (Atkin and Baker, 1987; Jenkyn and McCartney, 1987; Rodriguez et al., 1995; Dal Cin et al., 1998; Riopel et al., 1998). Using CGH, Kraggerud and colleagues confirmed that the most frequent change in dysgerminomas was gain of 12p, most commonly as i(12p) (Kraggerud et al., 2000). The frequency of 12p gain was distributed largely evenly amongst the age groups (12-32y). More recently, Cossu-Rocca and co-workers used fluorescence *in situ* hybridisation (FISH) to demonstrate chromosome 12p abnormalities in 17/21 (81%) dysgerminomas (Cossu-Rocca et al., 2006). The chromosome abnormalities observed included over-representation of 12p in 5/21 (24%) of cases, and i(12p) in 16/21 (76%) of cases. Patient ages ranged from 10 to 50y, although potential age-dependent variations were not considered. In conclusion, these results suggest that, similarly to males, 12p copy number gains may be associated

with increasing age in females, although more studies are required to confirm this, particularly in the TYA age group.

Other genomic copy number imbalances in MGCTs have also been described in adults. Commonly observed imbalances include gains on chromosomes 1, 7, 8, 12, 21, and X, as well as losses on 4, 5, 11, 13 and 18. However, none of these reported abnormalities are as consistent as gain of 12p (van Echten et al., 1995; Ottesen et al., 1997; Summersgill et al., 1998; Looijenga et al., 2000; Kraggerud et al., 2002; Ichikawa et al., 2013). A wide range of copy number imbalances has also been described in paediatric MGCTs, including gains on chromosomes 1q, 2p, 3, 7, 8, 13, 14, 20q, 21, and X, as well as losses on 1p36, 4q, 6q, 11, 13 and 18 (Perlman et al., 2000; Schneider et al., 2002; Oosterhuis and Looijenga, 2005; Palmer et al., 2007).

Susceptibility loci

Recent genome wide association studies (GWAS) have identified 6 susceptibility gene loci in the development of TGCTs in adults; *activating transcription factor 7 interacting protein* (*ATF7IP*), *BCL-2 antagonist/killer 1* (*BAK1*), *doublesex and mab-3 related transcription factor 1* (*DMRT1*), *KIT ligand* (*KITLG*), *sprouty homolog 4* (*SPRY4*) *and telomerase reverse transcriptase* (*TERT*) (Kanetsky et al., 2009; Rapley et al., 2009; Turnbull et al., 2010). However the specific mechanisms through which these genes result in tumorigenesis remain unknown. All six genes play a central role in normal PGC development (Gilbert et al., 2011) and, more specifically, may also be important in susceptibility to MGCTs in the TYA (*SPRY4, BAK1*) and paediatric (*BAK1*) populations (Poynter et al., 2012).

Protein-coding gene expression

One group studied the global messenger RNA (mRNA) protein-coding gene expression patterns of paediatric MGCTs, and compared them with published data on adult TGCTs (Palmer et al., 2008).

The results showed that paediatric MGCTs differed at the gene expression level according to their histology (Figure 2), irrespective of the anatomical site of the primary disease or the age of the patient (in the paediatric group). Similarly, adult TGCTs were also differentiated by their histology although, importantly, their gene expression profiles differed from their respective paediatric GCT subtype counterparts (Figure 3) (Palmer et al., 2008). These results demonstrated that paediatric and adult tumours with comparable histology can differ in their gene expression profiles, lending weight to the suggestion that the clinical management of these entities should be tailored accordingly. It is possible that differences in gene expression between paediatric and adult germinomas was attributable to contributions from tumour site in addition to patient age, given that the paediatric germinomas studied included both ovarian and extragonadal cases, which were compared to adult testicular seminomas. However, the fact that the YSTs were predominantly testicular in paediatric cases and that clear expression differences were still noted when compared to their adult testicular counterparts, makes a contribution from tumour site unlikely. This notion is supported by other studies, which suggest that site-related gene expression differences in GCTs are likely to be minimal (Korkola et al., 2009).

Korkola and co-workers also reported an mRNA gene expression signature that was predictive of TGCT clinical outcome in adult patients (Korkola et al., 2009). Genome-wide expression profiling was performed on 108 nonseminomatous GCTs from patients treated with cisplatin-based chemotherapy, 33 of which had poor clinical outcomes. More specifically, the results indicated that signatures reflecting enhanced immune function and repression of differentiation were associated with good outcome, while those representing active differentiation were associated with poor outcome, such that the gene expression signature derived added independent prognostic accuracy to existing clinically-used systems. Although patient age ranged from 15 to 65y (median age 29.4y) in the training set and 16 to 45y (median age 28.1y) in the validation set, the majority of cases were from older adults (>24y). Consequently, it is unclear whether this model could be applied directly to the paediatric and TYA setting. Large, future studies aiming to identify how such gene

expression profiles correlate with clinical outcome in paediatric and TYA populations (including direct comparisons with the above prognostic signatures for adult GCTs) are therefore required and should help to determine whether the use of transcriptomic signatures as adjuncts to clinical risk factors may inform treatment choice.

Non-protein-coding gene (microRNA) expression

MicroRNAs are short, non-protein-coding RNAs that modulate the expression of protein-coding genes through interactions with the 3' untranslated region (3'UTR) binding sites of target mRNAs, and whose expression profiles are dysregulated in cancer (Shenouda and Alahari, 2009). One group (Voorhoeve et al., 2006) demonstrated that the miR-371~373 cluster was highly expressed in adult TGCTs, suggesting that this may act as a potential novel oncogene system in TGCTs via inhibition of the tumour suppressor gene *LATS2*. Consistent with this observation, a subsequent study determined the levels of a restricted range of 156 microRNAs in adult MGCTs, and confirmed miR-371~373 over-expression compared with normal testis controls (Gillis et al., 2007).

Palmer and colleagues determined the global microRNA profiles of 48 paediatric and TYA samples (Palmer et al., 2010) and compared these with data from adult GCTs. The results showed that the majority of differentially expressed microRNAs in paediatric GCTs were down-regulated, consistent with observations in other malignancies. However, the most significant finding was that the miR-371~373 and miR-302 clusters were over-expressed in all MGCTs, independent of patient age (paediatric, TYA or adult), tumour histological subtype (YST/ seminoma/EC) or anatomical site (gonadal/extragonadal). Furthermore, the expression levels of eight main microRNA members from the miR-371~373 and miR-302 clusters accurately separated MGCTs from non-malignant samples, suggesting potential clinical use as highly sensitive and specific universal biomarkers of MGCTs (Figure 4) (Palmer et al., 2010).

Mechanisms for associations between age and GCT biology

Schneider and colleagues' study suggests a possible transition in biology and prognosis in younger versus older children, and proposes that this coincides with the increase in GCT incidence from 8y of age (Figure 1), although the aetiological factors responsible for this potential transition remain unknown (Schneider et al., 2002). One reasonable presumption is that the transition may follow endocrine changes linked to the onset of puberty. Increasing levels of oestrogen and testosterone associated with this event trigger the completion of the oocyte's first meiotic division and stimulate spermatocytes to enter meiosis, respectively. It is tempting to speculate that deregulations in this tightly orchestrated physiological process may in some way contribute to germ cell tumorigenesis. Thus, Schneider's study provides some genomic evidence pointing to a possible biological transition which may occur between GCTs arising in younger children ('paediatric-like') and those in older children ('TYA-like') and that such changes may, at least in part, be caused by the hormonal *milieu* surrounding the onset of puberty (Schneider et al., 2002).

Conclusion

We have reviewed the current knowledge regarding the biological similarities and differences in GCTs in paediatric and adult populations, and where GCTs in patients aged 13-24y may fit within this framework (summarised in Table 1). A persisting conundrum regards the mechanism and the timing of the 'tumour biological shift' between these populations. Should the inherent differences between paediatric and adult GCTs be accounted for by the onset of puberty, the majority of TYA GCTs may in fact be biologically more similar to adult tumours than previously thought (Schneider et al., 2002). Accordingly, we propose that future studies should correlate GCT biology with documented pubertal status (based on markers including steroid hormone levels and contralateral testicular size in males, as measured by ultrasound at the time of TGCT diagnosis) to investigate this theory. By contrast, there is no analogous physiological/developmental milestone that could

demarcate the presumptive transition between TYA and adult GCTs and their categorisation as distinct entities.

The equivocal boundaries between these groups are further complicated by age group-related differences in GCT clinical presentation and prognostic outlook, in turn reflecting variations in deliverability, tolerance and response to platinum-based therapies. In these cases, identifying tumour-specific biological characteristics (rather than simple patient age-based stratification) may be of greater value in prospectively identifying prognostic risk groups and support the development of novel, more targeted therapeutic regimes that have increased efficacy in poor prognosis tumours and/or cause less long-term toxicity in good prognosis patients.

As a result of these challenges, the authors have established a UK-based GCT consortium, which has generated a clinically annotated database supporting a tumour bank of over 400 MGCTs in patients within the TYA age range. The consortium proposes to utilise recent advances in technology to perform DNA, RNA and immunohistochemical-based studies on the associated paraffin-embedded MGCT tissues. Biological changes will be related to patient age at diagnosis across different histological subtypes and prognostic groups (including platinum-resistant cases), and compared with published results for paediatric and adult tumours. Both these and other future studies should aim to define age-specific GCT biological characteristics, including for TYA GCTs. Such an approach will help identify whether TYA GCTs represent a distinct clinical, physiological and biological entity, assist their optimal future management and may identify biological targets suitable for the development of novel therapeutic agents which may improve clinical outcomes for this under-investigated patient group.

Author Disclosure Statement

No competing financial interests exist.

Table and Figure Legends

Table 1: The biological characteristics of GCTs in paediatric, TYA and older adult age populations

Figure 1: GCT incidence rates by anatomical site, per million person years at risk, for patients aged 0-79y in England, 1995-2003 (from Murray et al., 2009).

Figure 2: Heatmap analysis of paediatric malignant GCT protein-coding gene expression data (from Palmer et al., 2008). The heatmap depicts all 657 differentially expressed probes in the 27 malignant GCTs analysed. In the heatmap, red represents relative mRNA over-expression and blue represents under-expression.

Figure 3: Heatmap comparison of protein-coding gene expression in paediatric malignant GCTs compared with pure (i.e. not mixed) adult malignant TGCTs (from Palmer et al., 2008). Twenty-seven paediatric tumours are compared with 20 adult malignant GCTs for (A) yolk sac tumours and (B) seminomas. In the heatmap, red represents relative mRNA over-expression and blue represents under-expression.

Figure 4: Differential expression of the miR-371~373 and miR-302 clusters in malignant GCTs (from Palmer et al., 2010). Hierarchical clustering analysis based on the eight main microRNAs from the miR-371~373 and miR-302 clusters segregates (A) paediatric and (B) adult malignant GCT samples from non-malignant controls (comprising benign teratomas and normal gonadal controls).

Table 1:

GCTs[a]	Age group	Biological Characteristics
Testicular	Paediatric	• Predominantly teratoma/YST[b] histology (Oosterhuis and Looijenga 2005) • Diploid (teratoma) or aneuploid (YST) (Oosterhuis and Looijenga 2005; Veltman et al., 2003) • i(12p)[c] is seen but to a lesser extent than in the TYA[d] population (Palmer et al., 2007) • Other chromosomal abnormalities include deletions of 1p, 4, 6q and gain of 1q, 12p13 and 20q (Oosterhuis and Looijenga 2005) • Overexpression of miR[e]-371~373 and miR-302 clusters (Palmer et al., 2010)
	TYA	• Predominantly nonseminomatous histology, having a median age of occurrence of 25y[f] (Oosterhuis and Looijenga 2005) • Aneuploid (Oosterhuis and Looijenga 2005; Veltman et al., 2003) • i(12p) is present in 80% of these tumours (Looijenga 2001) • Other chromosomal abnormalities include gain of 7, 8, 21 and loss of 1p, 11, 13 and 18 (Oosterhuis and Looijenga 2005) • IGCNU[g] seen in 90% (Dieckmann and Skakkebaek 1999; Jacobsen et al., 1981) • Overexpression of miR-371~373 and miR-302 clusters (Palmer et al., 2010)
	Older Adults	• Predominantly seminomatous histology, having a median age of occurrence of 35y (Oosterhuis and Looijenga 2005) • Aneuploid (Oosterhuis and Looijenga 2005) • i(12p) is present in 80% of these tumours (Looijenga 2001) • Other chromosomal abnormalities include gain of 7, 8, 21 and loss of 1p, 11, 13 and 18 (Oosterhuis and Looijenga 2005) • IGCNU seen in 90% (Dieckmann and Skakkebaek 1999; Jacobsen et al., 1981) • Overexpression of miR-371~373 and miR-302 clusters (Palmer et al., 2010)
Ovarian	Paediatric	• Incidence is very low before age 5y, then increases gradually to peak in early teens (Nicholson and Palmer 2010) • Predominantly teratoma/YST (Oosterhuis and Looijenga 2005) • Presence of i(12p) is infrequent, with only 4 cases reported (Bussey et al., 2001; Bussey et al., 1999; Speleman et al., 1990; Speleman et al., 1992) • Overexpression of miR-371~373 and miR-302 clusters (Palmer et al., 2010)
	TYA	• Incidence peaks in early teens (Nicholson and Palmer 2010) • Most commonly teratomas and dysgerminomas (Nicholson and Palmer 2010) • Diploid (Surti et al., 1990) • Overexpression of miR-371~373 and miR-302 clusters (Palmer et al., 2010) • i(12p) is present in a similar proportion to their testicular counterparts (Kraggerud et al., 2000)
	Older Adults	• Incidence decreases as age increases (Jha and Karki 2008)
Abbreviations	[a] Germ cell tumour, [b] Yolk sac tumour, [c] isochromosome 12p, [d] teenager and young adult, [e] microRNA, [f] years, [g] intratubular germ cell neoplasia unclassified.	

Figure 1:

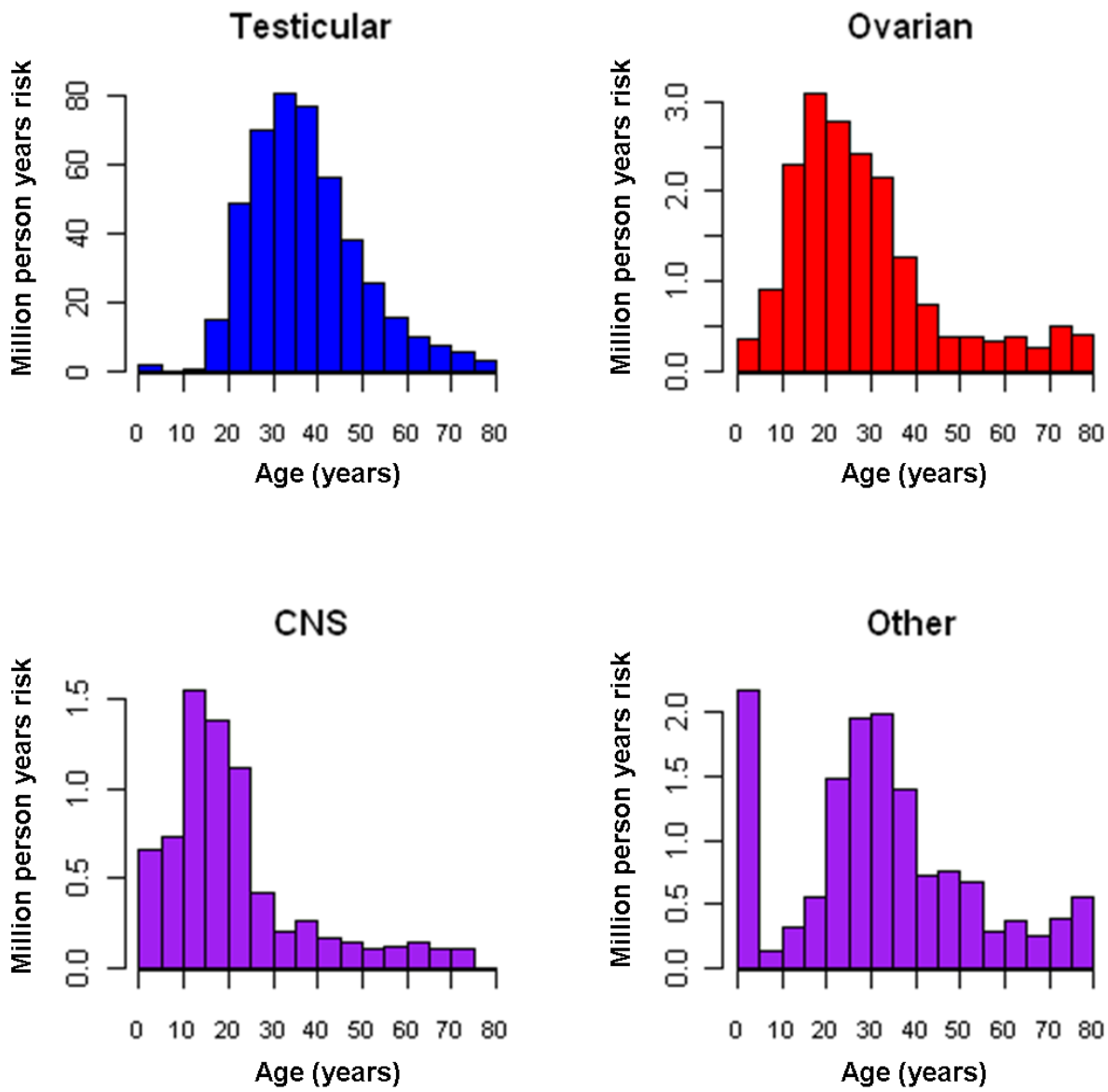

Testicular
Million person years risk
0 20 40 60 80
0 10 20 30 40 50 60 70 80
Age (years)
Ovarian
Million person years risk
0.0 1.0 2.0 3.0
0 10 20 30 40 50 60 70 80
Age (years)
CNS
Million person years risk
0.0 0.5 1.0 1.5
0 10 20 30 40 50 60 70 80
Age (years)
Other
Million person years risk
0.0 0.5 1.0 1.5 2.0
0 10 20 30 40 50 60 70 80
Age (years)

Figure 2:

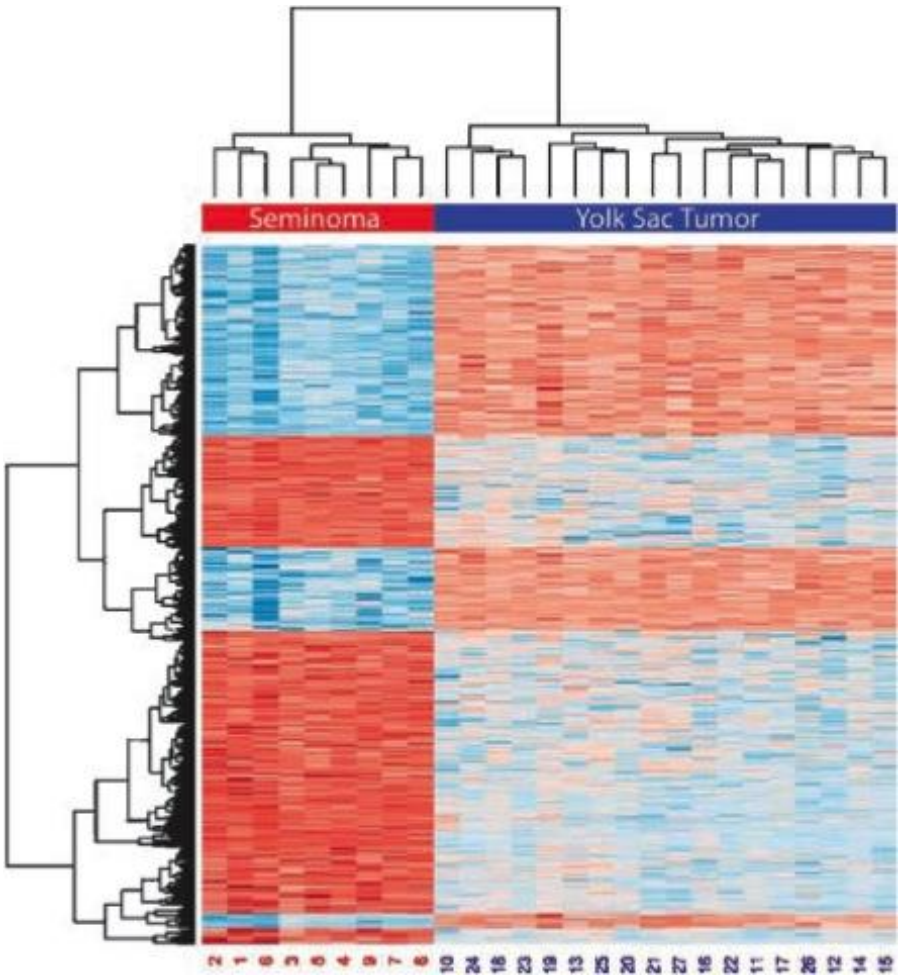


Figure 3:

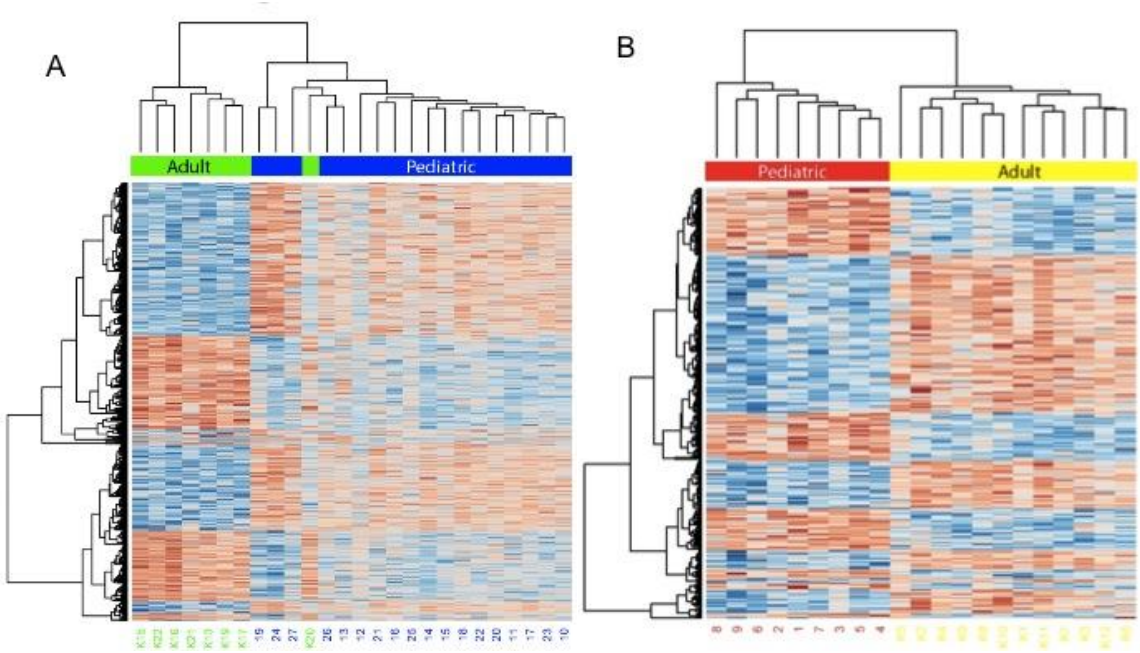
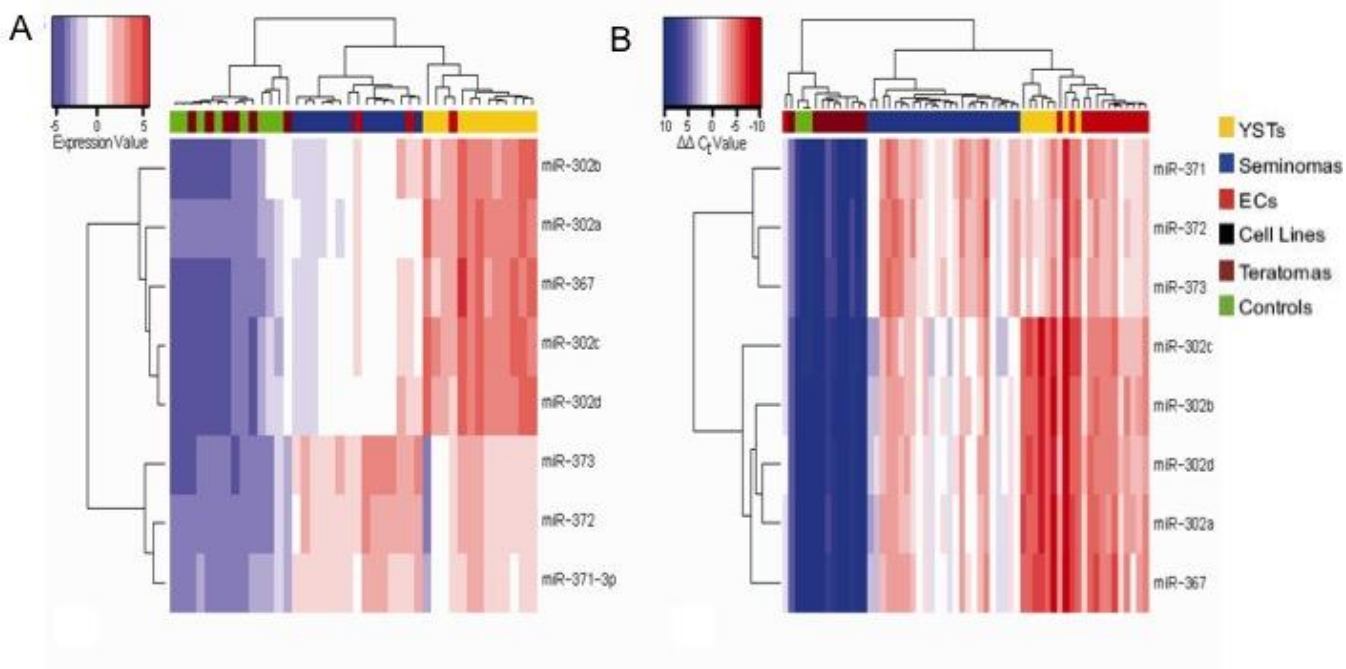


Figure 4:


A
-5 0 5
Expression Value
miR-302b
miR-302a
miR-367
miR-302c
miR-302d
miR-373
miR-372
miR-371-3p
B
10 5 0 -5 -10
ΔΔ Ct Value
miR-371
miR-372
miR-373
miR-302c
miR-302b
miR-302d
miR-302a
miR-367
YSTs
Seminomas
ECs
Cell Lines
Teratomas
Controls

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