TESTICULAR CANCER IN 2017

Sequencing advances understanding

Matthew J. Murray and Clare Turnbull

Our biological understanding of TGCTs has been improved using sequencing, and molecular profiles associated with the genomic evolution and development of cisplatin resistance have been identified. The genomics of variants underpinning TGCT predisposition is being delineated. Studies of circulating microRNAs have demonstrated their potential for noninvasive diagnosis and disease monitoring.

The incidence of testicular germ cell tumour (TGCT) is increasing and is a leading cause of death in young men. The introduction of cisplatin therapy 40 years ago resulted in improved survival for metastatic TGCT. Stratification of metastatic disease into good-risk, intermediate-risk, and poor-risk groups via the International Germ Cell Consensus Classification has, for the past 20 years, facilitated standardization of treatment and direct comparison of clinical trial results. However, escalation of therapy within the intermediate-risk and/or poor-risk groups has not generally resulted in clear survival advantages. Furthermore, attempts to identify additional clinical and/or tumour markers to predict platinum resistance have largely failed.

The end of 2016 saw publication of long-awaited landmark first insights into platinum resistance, afforded by large-scale agnostic next-generation sequencing (NGS). An analysis of 180 cisplatin-resistant and cisplatin-sensitive germ cell tumours, in which discovery whole-exome sequencing (WES) in 19 samples and targeted exon-capture-based sequencing of 300 genes in 161 samples was undertaken, was performed[1]. This study confirmed the low mean rate of small mutations (0.9/Mb) with the only frequent recurrent finding in TGCTs being gain of chromosome 12p (REF. 2). Distinguishing features of cisplatin-resistant compared with cisplatin-sensitive tumours were reported: elevated median number of small mutations and enrichment for *TP53* mutation or amplifications in the *TP53* regulator gene *MDM2*. Furthermore, WES was performed on 59 tumours from 51 patients with either platinum-sensitive or platinum-resistant tumours, including serial primary and metastatic sites where available[3]. Notably, all TGCTs had wild-type *TP53* with expression maintained in both primary and metastatic tumours. This report is the first to show a strikingly high frequency of reciprocal loss-of-heterozygosity copy-number-neutral events in TGCTs, which is much higher than other cancers, and which the researchers replicated in an independent series[2]. The hallmark trio of genomic characteristics in TGCTs were defined as wild-type *TP53*, chromosome arm 12p gain, and additional reciprocal copy number changes, and similarity to adaptive mutations acquired by human embryonic stem cells was highlighted[3]. The investigators hypothesized that the platinum sensitivity of TGCTs could be associated with high mitochondrial priming and demonstrated an increase in BIM BH3-induced mitochondrial depolarization in TGCTs[3]. Disappointingly, but probably unsurprisingly, these analyses did not converge upon a single neat biomarker of platinum resistance, nor a ready therapeutic target. However, together they offer biological insights into platinum sensitivity; additional depth may be gleaned from broader 'multiomic' profiling.

Improved detection and disease monitoring is another clinical priority. Conventional tumour markers (AFP, HCG, and LDH) have been used effectively for early risk stratification and detection of relapse in nonseminomatous GCTs but have limited sensitivity and specificity for patients with seminoma. Circulating microRNAs have had great promise as universal markers for diagnosis and disease monitoring for malignant GCTs since their first description in 2011 (FIG. 1) (REFS 4,5).

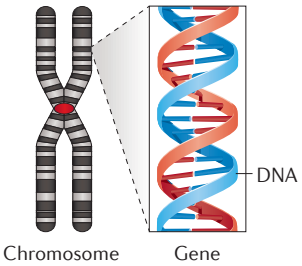
Using the highly sensitive multiplexed pre-amplification qRT–PCR technique[4], serum levels of a panel of four microRNAs (miR-371a-3p, miR-372-3p, miR-373-3p, and miR-367-3p) were analysed at primary diagnosis of 166 patients with TGCT and 118 men without[6]. Considerably higher serum expression levels for each of the microRNAs was seen in patients with malignant TGCT than in those without[6]. Notably, levels of miR-371a-3p fell to normal after successful completion of treatment, with persistently elevated values in patients who experienced treatment failure and relapse. Using the density estimation model, the sensitivity, specificity, and AUC for miR-371a-3p in the whole cohort were 89%, 93%, and 0.95, respectively, outperforming the conventional serum markers (which have a combined sensitivity of 50%)[6]. Analyses of three microRNAs (miR-371a-3p, miR-373-3p, and miR-367-3p) in 250 primary TGCT samples (seminomas and nonseminomatous GCTs) and 164 non-malignant samples[7] similarly showed the sensitivity, specificity, and AUC of these microRNAs combined to be 90%, 91%, and 0.96, respectively; for miR-371a-3p alone these parameters were 90%, 86%, and 0.95, respectively[7]. These studies indicate that miR-371a-3p might ultimately offer the most clinical utility, although

Key advances

- Large-scale exome sequencing studies of testicular germ cell tumours (TGCTs) revealed distinctive patterns of reciprocal chromosome loss, indicating potential mechanisms underpinning platinum sensitivity[1,3]
- Large-scale germline exome sequencing of multicase TGCT families revealed a polygenic genomic architecture underlying this disease[8]
- Genome-wide association studies[9,10] have doubled the number of identified common genetic variants linked to TGCT susceptibility from 25 to 49
- Biomarker studies demonstrated the superiority of a panel of circulating microRNAs (including miR-371a-3p) over traditional serum markers for TGCT diagnosis and monitoring[6,7]

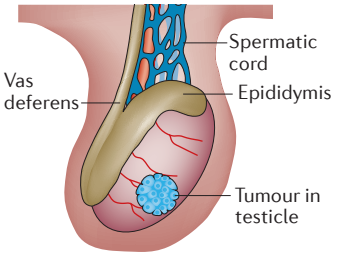
Genomic susceptibility

- Heritability is higher (49%) and familial relative risk greater (fourfold to eightfold) than for other cancers
- Susceptibility to testicular germ cell tumour (TGCT) is highly polygenic
- No major high-penetrance genes were found using exome sequencing of families
- Recent genome-wide association studies identified 49 common variants
- Implicated pathways include *KIT* signalling, transcriptional regulation of male germ cell development factors, and defective microtubule assembly



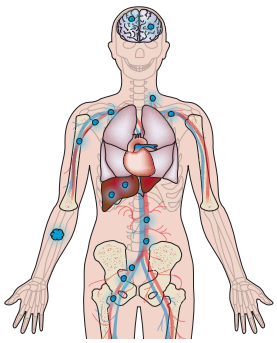
Somatic profiling of malignant tumours

- TGCTs have a low rate of somatic small mutations
- *KIT* and *KRAS* are the most frequent driver genes
- Wild-type *TP53* is typically retained
- A large number of copy number and structural variants are present in TGCTs
- i(12p) is the hallmark structural variant of TGCTs
- ***Features of seminoma:*** mutant *KIT*, hypertriploid
- ***Features of nonseminoma:*** wild-type *KIT*, hypotriploid
- Very distinctive patterns of copy-number-neutral reciprocal chromosome loss found in TGCTs



Understanding platinum sensitivity and resistance

- TGCTs are unusually and exquisitely platinum-sensitive (overall 5-year survival >95%)
- Mechanisms of platinum sensitivity are poorly understood
- The TP53 pathway (including MDM2) is likely to be important
- High mitochondrial priming could have a role in platinum sensitivity (as mitochondria have a high BCL2-related proapoptotic propensity)



Noninvasive microRNA monitoring for diagnosis and recurrence

- Current serum tumour markers (α-fetoprotein, human chorionic gonadotropin, and lactate dehydrogenase) have limited sensitivity and specificity, particularly for seminomas
- Quantitative real-time PCR of a panel of four circulating microRNAs is highly sensitive and specific for malignant TGCT diagnosis and/or disease-monitoring and relapse detection
- miR-371a-3p is the most individually predictive microRNA
- MicroRNAs could reduce the number of CT scans required in follow-up monitoring
- Prospective clinical trials are underway

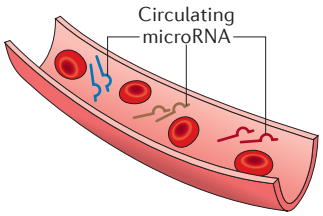


Figure 1 | **Testicular cancer in 2017 – advances in molecular understanding gained using large-scale sequencing.**

the rationale for assessing the full panel of all four in prospective trials has been highlighted[5]. These two large studies confirm previous reports demonstrating improved sensitivity and specificity of microRNAs compared with conventional serum markers. Validation in prospective clinical trials is under way, heralding an opportunity for noninvasive monitoring of malignant TGCTs and reduced use of serial CT scans and consequent radiation exposure[5] (FIG. 1).

TGCT has a strong heritable basis. In December 2016, the largest series of germline WES to date, comprising 328 TGCTs from 153 families experiencing multiple instances of TGCT, was published[8]. No gene was found in which disruptive mutations were segregating in more than 3 of 153 families. The top hit was *DNAAF1*, but with mutations only segregating in two families. Implication of *DNAAF1* in TGCT tumorigenesis was supported by demonstration of second-hit mutations and loss of protein staining for *DNAAF1* in tumours, as well as in a *DNAAF1*$^{hu255h(+/-)}$ zebrafish model. Mutations in six related genes were found in 9 of 151 further multicase TGCT families[8]. Larger WES studies are required, integrating familial and simplex cases. However, these results suggest that the contribution of rare alleles to TGCT heritability is highly polygenic. Based on these findings, clinic-based genetic testing for assessing inherited TGCT risk is unlikely to be useful.

Most of the high heritability of TGCT is likely to be underpinned by common genetic variants. Results of genome-wide association studies (GWAS) in TGCT have been remarkably fruitful, identifying variants with some of the highest effect sizes reported across all cancer types. In the past year, two major GWAS analyses[9,10] have resulted in an increase in the total number of identified TGCT-associated common variants from 25 to 49. By genotyping samples from 7,319 men with TGCTs and 23,082 controls, 19 new TGCT-associated loci were identified[9]. Widespread disruption of developmental transcriptional regulators, defective microtubule assembly, and dysregulation of *KIT–MAPK* signalling were revealed via chromatin-interaction analysis as potential mechanisms key in early oncogenesis. A meta-analysis of five GWAS for TECAC[10] identified eight TGCT susceptibility loci, of which five were novel and three overlapped with those identified in the other GWAS[9,10] (FIG. 1).

The growing number of rare and common susceptibility alleles is complementing our expanding landscape of molecular determinants of TGCT development and treatment response. Translation of our advances in molecular understanding of TGCTs into clinical benefit for patients is the next challenge.

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Competing interests statement
The authors declare no competing interests.