Chapter 45
What do we know about the origin of germ cell tumours?

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Why is it important to understand this?

Testicular germ cell tumours are the most commonly diagnosed solid tumour in 15-44 year old men, ranging in incidence from 1:1000 to 1:100,000, depending on location. In Chapter 44, the clinical importance of testicular germ cell tumours (TGCTs) to the reproductive health of young men was presented. There is a good prognosis with a >95% 5-year survival rate in men being diagnosed at an early stage in the US. Upon improvement of non-invasive diagnostic tools, early diagnosis will become easier. However, if the tumour has spread or is refractory to therapy, the picture worsens significantly to 75% survival or below. The added burden of co-morbidities and associated risk of infertility linked with a TGCT diagnosis are of concern to patients, their families and health care providers. The risk of poor outcomes for men with this disease will be increased in settings of limited medical consultation or in the absence of encouragement to seek an early diagnosis when a testicular lump is detected.

In addition to the potential of genetic risk factors to impair adult male reproductive health and testis function, accumulating evidence shows that early life exposures, particularly those *in utero*, have a substantial impact. TGCT incidence is increasing in many parts of the globe, particularly where environmental stressors (e.g. industrialisation, lifestyle factors) and genetic risks are at play. Rates of increase of 2% per annum and higher are reported for many countries. In Australia, the age-standardized rate increased from 4.2 cases to 7.5 per 100,000 males between 1982 and 2017; in several European countries, including Germany, an increase of nearly 3% per annum is predicted, while those with the highest rates (e.g. Denmark) are expected to experience a decline. These numbers highlight the need to learn what contributes to TGCT risk that cannot be attributed to genetic factors. Alongside the higher TGCT incidence is the escalating prevalence of associated testicular dysgenesis

pathologies, including male infertility, hypospadias and cryptorchidism. The accumulating evidence that tumours within other organs arise from male germline cells provides additional clinical relevance to understanding the genesis and outcomes of inappropriate germ cell development.

Clinical and biomedical research in Andrology that builds knowledge of TGCT origins will be relevant to developing approaches to limit both its incidence and spread. The long-standing goal is to provide evidence of how to reduce TGCT incidence and testicular dysgenesis through changes in lifestyle, manufacturing and medical practices, and ultimately influence public policy.

The fundamental information

Although direct observations are lacking, human germline cells are considered to be specified during the second week of gestation (GW2) as primordial germ cell (PGC) precursors, under the influence of bone morphogenetic proteins (BMPs), Activins and WNT signalling proteins. Moving to the extraembryonic endoderm, they appear as large rounded TNAP (tissue non-specific alkaline phosphatase)-positive cells with a large nucleus and clear cytoplasm. This physical separation of PGCs from somatic cells is required for their unique developmental trajectory; near-complete erasure and replacement of genomic epigenetic marks involving DNA methylation is essential to form gametes that can produce healthy offspring (Chapter 13). The sexually indifferent PGCs migrate to the gonadal ridge by ~GW5. In nascent testes, somatic cells expression of *SRY* establishes male fate by GW8. Now termed gonocytes or pre-spermatogonia, germ cells become enclosed into cords by Sertoli cells, in an epithelium that will surround and support spermatogenesis throughout life. In rodents, gonocytes proliferate, then enter a key phase of quiescence and epigenetic reprogramming (re-methylation) spanning one week; after birth they resume proliferation, migrate to the cord perimeter, and differentiate into spermatogonia. In contrast, human gonocytes do not develop as a single, synchronous population. By ~ GW13, some move from the cord centre to perimeter, displaying decreased OCT4 and increased NANOS2 and DDX4 levels. This transformation of gonocytes into prespermatogonia proceeds through the 2nd trimester and is accompanied by cell cycle exit, with this transformation complete by the hormonal surge of mini-puberty occurring by 4-6 months postpartum.

As the embryo develops, the various testicular somatic cells multiply and differentiate, and immune cells enter the testis. An important period of embryonic development involves the synthesis of testosterone (T) at levels that drive masculinization. Fetal testis steroid production involves both Leydig cells that convert cholesterol to androstenedione (A4) and Sertoli cells that convert A4 to T. The key window of masculinization in human embryos (~GW8-14) marks the period when disruptions to normal steroid production can derail the normal developmental trajectories of the testis, the male reproductive tract (e.g. reduced anogenital distance), and potentially influence other organs.

Understanding TGCT origins

Successive observations by clinician-scientist Niels Erik Skakkebaek yielded the first evidence that TGCTs develop from fetal germline cells that persist in the adult testis. He documented histological observations from two men presenting with infertility. The initial biopsy for each showed abnormal tubules containing only Sertoli cells and mitotically active, possibly tetraploid, cells appearing similar to spermatogonia but with enlarged nuclei. Subsequent biopsies revealed embryonal carcinoma (EC) detected 16 months later in one man, and atypical germline-like cells 4.5 years later in the other. These findings revealed that the cells we now term germ cell neoplasia *in situ* (GCNIS; formerly named carcinoma *in situ*, or CIS cells) progress to form the two main TGCT subtypes: non-seminoma (EC-like) and seminoma (similar to gonocytes).

Subsequent decades of histological and molecular analyses provided strong evidence that TGCTs arise from fetal germline cells that do not differentiate properly and are not eliminated by apoptosis. Robust markers of germline differentiation status illustrate that the expected trajectory of progressive transcription factor expression does not take place in these cells. Many markers found in human primordial germ cells and gonocytes are present in GCNIS cells, and persist in seminomas, reflecting the gonocyte-like phenotype of these tumours and the repression of differentiation. These include *PRDM1*, previously named *BLIMP1*, a hallmark transcriptional repressor protein in germline cells, in addition to *POU5F1* (encodes OCT4), *LIN28*, and *NANOG*. *SOX17* is present in PGCs, gonocytes and seminomas, but absent from non-seminomas which in contrast express *SOX2*, a direct target of PRDM1, as an indicator of their more differentiated status; both of these can

heterodimerize with OCT4 to maintain pluripotency. Non-seminomas also express pluripotency markers, *POU5F1, LIN28*, and *NANOG*, in addition to *GDF3, TDGF1* (encodes CRIPTO), and *DNMT3B*, a profile which aligns them to embryonic stem cells.

Genome-wide association studies (GWAS) have identified many candidate mutations for TGCTs, but no driver mutation for all subtypes, highlighting the expectation that environmental factors affecting *in utero* development provide the key to understanding the risk of GCNIS cell formation that underpins the growing incidence of TGCT. GCNIS and their derivative TGCT cells are typically aneuploid. The most common genetic rearrangement in GCNIS cells that progress to TGCTs is isochromosome 12p gain; mutations in *KIT* (encodes a signalling receptor), *KRAS* and *NRAS* (encode signal transduction proteins) and CpG island undermethylation are hallmarks of seminomas, while hypermethylation (associated with gene silencing) of genes associated with DNA repair, is documented in non-seminomas. These differences in methylation indicate that there are several mechanisms or routes by which these cells become vulnerable to disruption.

Disruptions to masculinization programming

Disruption of steroid hormone activity in the masculinization programming window (MPW) affects the male germline; the timing of vulnerability in rodents (rat, embryonic day [E]15.5-18.5, mouse, E13.5-E15.5) coincides with the lowest genomic methylation levels and when gonocytes become (and are) quiescent. The human MPW is considered to occur from GW8-14 in fetal human testes.

Steroid disruptions in the MPW affect somatic cell functions, resulting in focal areas with fetal Leydig cell aggregations and Sertoli cells outside cords; such changes could influence the germline indirectly. In rodent *in vivo* and *in vitro* studies, germ cells exposed to phthalates can appear as multinucleated and also outside of the cords. However, evidence from studies of mice lacking normal androgen receptor function demonstrate that germ cells may be directly affected in the absence of appropriate stimulation, with some delay in entering quiescence. Altered levels of testosterone as well as estrogens can reduce germline differentiation and survival during this crucial window.

Formation of the male phenotype requires differentiation of both internal and external genitalia. In rodents, this is stimulated by fetal testis-derived androgens,;however in humans, placental-derived hCG

and androsterone are synthesised in the placenta, and fetal liver and adrenal gland also produce androgens. Testes from rodents exposed to endocrine disrupting environmental chemicals, such as dibutyl phthalate (DBP), exhibit changes that simulate phenotypes seen in humans, including reduced anogenital distance in males, cryptorchidism, hypospadias, and reduced fertility. This spectrum of phenotypes, collectively termed Testicular Dysgenesis Syndrome (TDS) (Chapter 44), may be mechanistically linked due to their co-association, including with increased risk of TGCT formation. For example, cryptorchidism is linked with a 4-fold greater risk of testicular cancer.

What pathways, cells, and factors contribute to GCNIS?

How GCNIS cells emerge and persist in the fetal human testis cannot be directly determined for ethical and practical reasons, and no experimental animal models reliably recapitulate this pathology. A highly regarded concept is that two stages of germline changes are required to generate TGCTs, and the extended period of human germline quiescence (spanning 1 or more years) is permissive, in contrast to the week interval of germline quiescence in rodent models. Cultures of fetal human testes have been used to validate hypotheses regarding what signalling pathways are involved when the somatic cell environment facilitates survival and/or proliferation of neoplastic germ cells. Fragments (1^{st} and 2^{nd} trimester) exposed to the signalling protein NODAL contained greater numbers of OCT4+ cells, although this pathway activity and this marker is normally downregulated as gonocytes transition into pre-spermatogonia. The NODAL co-receptor, CRIPTO, is a biomarker for TGCTs that can be detected in the serum of men with GCNIS cells, correlating positively with a second biomarker, miR-371a-3p.

The future

Simultaneous interrogation of multiple markers may eventually indicate tumour cell identity and status, to guide appropriate interventions. Roles and responses of other somatic cells, especially immune cells, are under investigation for their roles in fetal testis growth and TGCT aetiology. This fundamental research should one day explain why some immature germ cells are inappropriately

retained in the adult testis, and what governs their transition into neoplastic cells.

Suggested reading

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