ABSOLUTE POWER CORRUPTS ABSOLUTELY: INSIGHTS TO TESTICULAR GERM CELL TUMORIGENESIS

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Abstract: Testicular germ cell tumors are the most frequent malignant tumors of young males (age 15 to 35). While the causes of their malignant transformation are still unclear, the two main tumor groups (seminomas and non-seminomas) share a common origin and distinct biological pathways. The pluripotence of germ cells explains their differentiation in various histological subgroups (classic seminoma, spermatocytic seminoma, embryonal carcinoma, choriocarcinoma, yolk sac tumors, teratomas and several mixed-type tumors). It may also be the reason behind the extraordinary response to oncologic treatments (chemotherapy in particular) of these tumors, representing a model of curable cancer from which oncologists and researchers can draw future insights to the optimal management of other neoplasms.

INTRODUCTION

Testicular germ cell tumors (GCTs) have a low frequency (1% of all male cancers), but nevertheless represent a public health problem in that they have a unique epidemiologic profile amongst solid tumors. Their incidence peaks at ages between 25 and 35 years, and also displays a distinctive racial and geographical distribution – higher frequency in the Caucasian population of Northern Europe and the United States (risk of occurrence of about 1 in 400 males).

While raw incidence rates in Europe have constantly increased during the 20th century, now being about 4.8/100 000 per year, survival rates have improved steeply from around 60% in the 1960s to date, when mortality is at a low 0.65/100 000 per year. This huge chance of obtaining a cure (of 92-95%, and 75-80% even in metastatic disease), the highest among all solid tumors, is certainly due to (not-so-)modern chemotherapy protocols (mainly, the advent of cisplatin in the late 1960s), but also to retroperitoneal surgery, progress in imaging techniques, and individualized risk assessment.

A correct, complete and standardized (or centrally reviewed) pathology examination, strict surveillance of serum tumor markers, as well as immunohistochemical and genetic evaluation of newer markers, could theoretically maximize response rates and survival. Therefore, achieving substantial mitigation of therapeutic side effects by avoiding “overtreatment” in selected cases, while not compromising the chance of cure for others, has currently become a constant concern for urologists, medical and radiation oncologists; the psychological, social and economic impact of curing (but also following-up) these patients (the majority of which are young) is enormous. The extensive studies on prognostic factors allowed not only for treatment personalization, but also a better understanding of the natural history of GCTs (and even extrapolations to other solid tumors). However, data on genetics and biology of GCTs are still scarce.

Clinically, GCTs are a heterogeneous group of neoplasia, localized predominantly in the testis (rarely in the retroperitoneum, mediastinum, or pineal gland). Histologically, they are classified as seminomas and non-seminomas. The former do not differentiate and retain characteristics of germinal cells; currently, their end-stage character is subject to controversy, and their ability to transform into non-seminomas remains subject to speculation in the absence of clear evidence. Non-seminoma precursors share a remarkable ability to differentiate *in vivo* towards either embryonic (embryonal carcinoma, EC), extraembryonic (yolk-sac tumors, YST, and choriocarcinoma, CC) or somatic components (mature and immature teratoma, MT/IT).

Although in an abnormal, spatially and temporally uncoordinated manner, GCTs follow models of differentiation mimicking the stages of development of the normal zygote. Thus, seminomas are thought of as originating in transformed germinal cells, which divide by mitosis and have conserved the inhibitory mechanisms responsible for the zygote-like differentiation. Such perspective is endorsed by genetic and immunohistochemistry studies, which showed, e.g. expression of c-kit (CD117, normally present in spermatogonia and order I spermatocytes), of the stem cell growth factor (SCF), and the absence of cytokeratins. On the other hand, c-kit is not expressed in most non-seminomatous GCTs, while SCF is overexpressed and low molecular weight (MW) cytokeratins can be identified; this pattern confirms the loss of the germinal phenotype and the occurrence of features which allow for somatic differentiation of the tumor cells. The major gap between seminomas and non-seminomas – determining the biologic evolution, treatment response and different prognosis – is loss of the capacity to conserve totipotence by the latter.

With respect to their biology, GCTs can be thought of as one of nature’s microlaboratories, in which multiple evolutionary possibilities are “studied”. By their intrinsic nature, germ cells are truly omnipotent (as are stem cells), feature which is normally activated by fertilization; proliferation, survival and retention of “omnipotence” by these cells are regulated by their microenvironment, through multiple stimulating/inhibiting cytokines, growth factors, receptors and intra-/intercellular signaling pathways.

HISTOGENESIS OF TESTICULAR GERM CELL TUMORS

Several initially abandoned theories have been rehabilitated recently. Basically, the current concept is that all GCTs (with the possible exception of spermatocytic seminoma, SS) fundamentally root in a pluripotent germ cell and that the tumorigenesis process is multistage and affects one of the major germ line differentiation pathways. As such, GCTs would be progressing from a carcinoma *in situ* (CIS, better termed intratubular germ cell neoplasia, ITGCN), which in turn comes from the processing gonocytes. It appears that malignant transformation affects germ cells during migration, hence germinal tumors may occur either in the gonads, or in extragonadal sites.

Histological tumor type depends on the differentiation step the germ cells are on when malignant transformation occurs: seminomas are derived from primordial germ cells, ECs arise while embryonic differentiation is ongoing, and YSTs or CCs appear by extraembryonic differentiation; teratomas, which mostly have a benign behavior, arise when full differentiation has already taken place [Bosl *et al.,* 2008. Brandli *et al.,* 2003]. Therefore, seminomas are less differentiated primitive tumors reflecting gonadal differentiation; differentiation is stopped later on in EC, an aggressive tumor composed of cells with pluripotent differentiation capacity to one of two directions. Teratomas may reflect either the embryonic stage (IT) or somatic differentiation (MT), containing all three primitive embryonic layers: ecto-, endo-, and mesoderm. On the extraembryonic differentiation pathway, malignancy seems to recreate placental trophoblast development (CC), or resembles the meso- and endodermic tissues found in the second week blastocyst (YST), which should subsequently disappear. CCs, YST and teratomas are considered partially differentiated (conserving 'normal' embryogenesis), while EC is seen as the least differentiated non-seminomatous GCT [Looijenga and Oosterhuis, 2002].

Although this general pattern appears to be widely accepted, supported by histopathology and experimental studies, some clinical observations and evidence bring into question the universality of this theory. Mixed-type tumors (seminoma plus non-seminomatous components), the association of isolated syncytiotrophoblastic cells with seminoma, or development of non-seminomatous metastases in patients with pure seminomatous testicular primary has suggested that further differentiation potential exists, at least for seminomas. Immunohistochemical studies, cytology and flow cytometry provide more arguments for this possibility. On the other hand, identification of *in situ* non-seminomatous elements in the seminiferous tubules supports the hypothesis that – sometimes – differentiated tumors may occur directly, without passing through "intermediate" phases such as seminoma or EC [Bosl *et al.,* 2008. Richiardi *et al.,* 2002.].

GENETICS AND BIOLOGY OF GERM CELL MALIGNANT TRANSFORMATION

Cytogenetic studies (cariotyping, fluorescent/chromogenic *in situ* hybridization – FISH/CISH, spectral cariotyping – SKY etc.) of GCTs in short-term cell cultures identified the primordial cell, demonstrating a complex, but very similar genetic structure of seminomas and non-seminomas (partial underexpression of chromosomes 4, 5, 11, 13, 18 and Y; overexpression of chromosomes 7, 8, 12 and X). GCTs are invariably XY (indicating their origin in a premeiotic or meiotic germinal precursor, before anaphase I) and are always aneuploid (related to centrosome amplification). Seminomas and their original lesion – ITGCN – are hypertriploid, while non-seminomas are hypotriploid without regard to their histological subtype. This might suggest that polyploidization could be the triggering event to determine the occurrence of a tetraploid ITGCN, followed by net loss of chromosomal material [Port *et al.,* 2005].

The first GCT genetic marker to be identified was the isochromosome of the short arm of chromosome 12 [i(12p)] (Atkin and Baker, 1982). Subsequently, several researchers have argued that the i(12p) is an unique chromosomal marker for all histological types of GCTs, in all primary tumors and 80% of GCTs growing in short-term cell cultures; its presence was also revealed in ITGCN, as a very early mutational event in the malignant transformation of the GCTs (but it has not been shown to be a cause of the process) [Rodriguez S *et al.,* 2003].

GCTs have been found to express an increased number of copies of genetic material from the 12p region, suggesting that gene amplification or modification located on this chromosome play a major role in the development of malignancy. Because of the great specificity of i(12p), it can be an useful genetic marker in addition to histological diagnosis of less differentiated tumors, such as those located in the midline of the body in people aged under 50 years (extragonadal germinal tumor syndrome, EGTS).

The 20% of GCTs not containing isochromosome 12, also called i(12p)-negative tumors, present chromosomal damages with aberrant band markings. Further studies indicated that these chromosomes contain 'in tandem' duplications of material from the 12p portion, which nevertheless supports the hypothesis that one or more genes located here are intimately involved in the malignant transformation of germ cells. A second substantial change in chromosome 12 is deletion affecting its long arm, which was reported in up to 20% of GCTs [Rodriguez F *et al.,* 1993].

Deletion anomalies are a classic indicator for loss of (function of) tumor suppressor genes (TSGs), recessive genes that normally inactivate the structural (dominant) ones controlling cell growth, as demonstrated in retinoblastoma (Rb1 gene), Wilms' tumor (WT1 gene) etc.

A member of the D class of cyclins (substances that propel cells into the cell cycle) – cyclin D2, involved in regulating the transition (checkpoint) of the G1 phase to the S phase of the cell cycle – is encoded by a gene located in the

12p portion and is overexpressed in virtually all GCTs; the hypothesis that this is an oncogene involved in the development of GCTs is under study in transgenic mice. Another candidate that could explain the gain of 12p genetic material is the K-ras2 gene. Cytogenetic studies of GCTs also raised suspicion of proto-oncogene activation by amplification of one or more genes probably located in the 12p11.2-p12.1 region, loss of TSGs on chromosomes 12 and 1, and/or other non-random chromosomal alterations. Practical reasoning – chromosome gain should be associated with increased copy numbers of the dominant promoter genes, while chromosome loss would be associated with TSG loss – is difficult to apply here, given the polyploid nature of the GCTs [Schmidt *et al*., 2001. Murty and Bosl, 1994].

Table I. *Frequency of non-random chromosomal abnormalities in GCTs* [Murty and Chaganti, 1998]

Altered chromosome	Anomaly type	Frequency (%)
18	loss / monosomy	56
13	loss / monosomy	55
11	loss / monosomy	52
5	loss / monosomy	52
4	loss / monosomy	47
9	loss / monosomy	39
21	gain / trisomy	35
8	gain / trisomy	32
12	12q12-q24 deletion	20
7	7q11-q36 deletion	20
1	1p21-p36 deletion	16
6	6q14-q25 deletion	12

Structural anomalies were recorded only if over 10% in frequency and/or 30% in number. Gain or loss was reported over the normoploid chromosome number.

Specific chromosomal deletions have led to the discovery of TSGs in several cancers. Well-known suppressors (p53, Rb1), but also genes deleted in other cancers (DCC, NME), were surveyed in GCT oncogenesis.

P53, located in region 17p13.1, is the most frequently deleted / mutated gene in a variety of tumors. It encodes the synthesis a cytoplasmic protein (MW 53kDa) essential for many cellular functions, including regulation (through another protein, p21) of cell transition from the G1 to the S phase of the cell cycle, promotion of DNA integrity, inducting repair mechanisms and hindering cell entry into S phase [Scherr, 1994].

The p53 gene acts as a transcriptional mechanism regulating multiple genes through direct binding to DNA. Another extremely important function of p53 is the regulation of apoptosis (programmed cell death) in cells with DNA damage (can initiate interruption of the cell cycle, thus allowing the repair of DNA lesions, or trigger apoptosis if repair is not possible). P53 mutations (seen in over 50% of human malignant tumors) can cause loss control over the cell cycle and apoptosis, finally resulting in malignant proliferation. Identification of genome-wide alterations by allelotypic measuring of the loss of heterozygosity (LOH) in multiple chromosomal loci, allowed linking the molecular lesions (e.g., loss of chromosomal loci) to clinical development and discovery of novel sites for candidate oncogenes. Applying this method to GCTs (currently focused on certain parts of chromosomes 1, 3, 5, 11, 12 and 18) showed that p53 does not appear as mutated, and that the existing LOH in the 17p13.1 region could be a deletion accompanying another target gene. Male GCTs also express high levels of wild type p53 protein, which could explain their particular sensitivity to cisplatin-based chemotherapy. Emergence of p53 expression mutations is supposedly associated with the occurrence of cisplatin resistance, but these situations are rarely concomitant in GCTs. Also, in F8 murine cell lines and human GCTs exposed to etoposide, a substantial increase in p53 protein (and subsequently apoptosis) was reported [Guillou *et al.*, 1996].

The role of another important cellular suppressor, the retinoblastoma-1 (Rb1) gene, was also studied in GCT tumorigenesis. The Rb1 gene (currently seen as the prototype of TSGs) is located in the 13q14-32 region; its product is a phosphorylated nuclear protein (MW 105-110kDa) that acts in association with cyclin D by initiating the transition from the G1 to the S phase of the cell cycle. Loss of Rb1 gene function by deletions and/or mutations causes a disturbance of the G1 phase and cell cycle disruption. Besides retinoblastoma, Rb1 is involved in a variety of tumors (breast, bladder, small-cell lung cancers and sarcomas).

Cytogenetic studies have identified abnormalities of chromosome 13 (monosomy / absence) in over 50% of GCTs; LOH analysis revealed deletions of alleles in the 13q14-32 region with almost the same frequency. For example, two different studies have identified 13q14 deletions of Rb1 in 30-39% of the studied GCTs, suggesting that loss of Rb1 function may play an important role in the pathogenesis of GCTs. Evaluation of Rb1 protein expression led to the finding of a 3 to 15 times decrease in most GCTs, for all histological subtypes, compared with the level of expression in normal testicular tissue. These studies have suggested the possibility of two different operating mechanisms of the Rb1 gene in GCTs: by loss or disruption of gene function and by increasing initiation of Rb1 transcription in differentiated cells. However, Rb1’s exact mechanisms of action in GCTs need further clarification [Murty and Bosl, 1994. Peng *et al.*, 1995].

Another suppressor gene, originally identified in colorectal cancers, the DCC (Deleted in Colon Cancer) gene, has been discovered in GCTs, breast, stomach, pancreas, prostate cancer, leukemia and glioblastoma. Located in region 18q21.3, it encodes a protein similar in structure to neural cell adhesion molecules (NCAM), involved in intercellular and cell-basal membrane interactions. In GCTs, underexpressed chromosome 18 was found in over 50% of cases and the 18q21 region frequently expresses heterozygosity loss and intragenic deletions in 45-55% of tumors. LOH and loss of DCC expression are characteristic for both primary and metastatic GCTs, suggesting that genetic alterations of DCC are an early event in the development of these tumors. Also, patients with deletions of the DCC seem to have an unfavorable prognosis [Strohmeyer *et al.*, 1997. Murty *et al.*, 1994].

The NME (Non-Metastatic Enzyme) genes 1 and 2 encode a structural protein (MW 23 kDa) for an enzyme called nucleotide-diphosphonic kinase (NDPK), originally identified in melanoma cell lines; their high expression was inversely correlated with progression to metastatic phenotype of certain tumors, thus inferring their function as metastasis suppressor genes. Levels of mRNA expression and enzymatic activity of NDPK in some tumors are high as compared with normal tissues; also, structure similarities between NME and gene AWD of Drosophila were observed, which led to the conclusion that NME are involved in regulating cell differentiation. Genes NME-1 and -2 are frequently deleted, and proteins NDPK-A and NDPK-B are 4-5 times less expressed in teratoma, as compared to EC. These data suggest that NME genes may play a suppressive role in somatic differentiation of GCTs [Baker *et al.*, 1994].

Random genetic variation composing the replication error phenotype (RER) is usually detected as microsatellite instability (MSI). This genetic mechanism is associated with the development of several tumors, such as hereditary non-polyposis colorectal cancers (HNPCC, Lynch syndrome), and includes a family of genes responsible for repairing DNA errors, such as MSH2 (2p22), MLH1 (3p21), MSH6 (2p16), PMS1 (2q31-q33) and 2 (7p22), TGFBR2 (3p22) and MLH3 (14q24.3). RER-type instability was identified in GCTs, but less strongly than for HNPCC. Genomic instability in the 1q42-43 region has been reported in less differentiated GCTs (EC, YST) more than in teratoma [Murty *et al*., 1994].

Involvement of the structural gene (proto-oncogene) compartment in malignant transformation of testicular GCTs has been less well researched. Cytological studies have shown the presence of proto-oncogene amplification by homogeneous staining reaction (HSR), 'double-minute' and chromosomal banding aberrations. Other researchers have failed to identify amplification of known oncogenes in GCTs, suggesting that a new type of gene can be amplified in this tumor type. Increases in the copy number of oncogenes coding for platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), but not changes in mRNA levels, have been highlighted [Skotheim *et al.*, 2002].

The c-kit proto-oncogene, which encodes a membrane tyrosinkinase, is located in the same region as the PDGF receptor (4q12), both sharing high structural uniformity. The c-kit product plays a crucial role in the development of pluripotent hematopoietic stem cells, and also in melanoblast and primordial germ cell migration during early embryonic development. The c-kit oncogene ligand and a growth factor of mast cells (MGF) are located on the 12q22 portion. Byproducts of both genes, c-kit and MGF, are essential for embryonic development of mammalian germ cells. Two separate studies have established that the oncogene c-kit is expressed in most seminomas, while MGF levels are higher in non-seminomas. Another gene encoding a growth factor is HST-1, which belongs to the family of fibroblast growth factors (FGF) and expresses similar characteristics as MGF [Palumbo *et al*., 2002].

These studies showed that the majority of known oncogenes are not amplified in GCTs, and expression of genes for several growth factors is related to a specific cell line during differentiation. Other assumptions regarding the molecular events underlying GCT malignant transformation, differentiation, and sensitivity to chemotherapy remain to be proven.

In light of current data, carcinoma *in situ* (CIS) of the testis is considered a precursor stage for both seminomatous and non-seminomatous GCTs (except SS in adults, and YSTs in children). This atypical intratubular germinal tumor was associated with subsequent development of invasive cancer; CIS foci were found in proximity to most germinal tumors at pathologic examinations [Skakkebaek, 1972]. The term CIS is actually nosologically incorrect because it implies an epithelial origin. Later, the name intratubular germ cell neoplasia (ITGCN) was coined, and the term 'unclassified' (ITGCNU) may be added to distinguish forms of specific, recognizable intratubular differentiation (such as seminoma, EC, and even CC) from the much more common undifferentiated forms [Dieckmann and Skakkebaek, 1999].

After Skakkebaek's initial communication, other observations have reinforced the belief that ITGCN is a precursor of invasive GCTs. These included an increased incidence of ITGCN in patients with a history of cryptorchidism (3-8%, with a 5% risk of developing invasive cancer in the first five years after ITGCN diagnosis), testicular cancer (5% in the contralateral testicle), infertility, gonadal dysgenesis, intersexuality or frank hermaphroditism [Ramani *et al.*, 1993]. It is assumed that CIS cells differentiate from primordial germ cells during embryogenesis (possibly due to an excess of estrogen), and remain dormant in the seminiferous tubules until puberty [Morton and Thomas, 2004].

Comparative studies on membrane proteins (e.g., PLAP, c-kit), germ cell markers (e.g., MAGEA4, VASA, TSPY, NY-ESO-1) and cell cycle regulatory proteins (e.g., p53, P19-INK4d, CHK2) showed similarity between ITGCN, primitive germ cells and gonocytes, supporting the hypothesis of the premeiotic origin of ITGCN; recent studies on gene expression profiling, using microarray technology, showed that they are also very similar to embryonic stem cells, but failed to identify patterns of gene polymorphisms that may predispose to the emergence of GCTs.

The most likely pathogenic mechanism of ITGCN is impaired gonadal development, resulting in arrest of gonocyte differentiation and maintenance of their embryonic characteristics, associated with increasing genomic instability; amplification of certain chromosomal regions (e.g., 12p) would then facilitate cell survival, circumvention of apoptosis mechanisms and subsequent progression to invasive phenotype. Association of ITGCN with other disorders (genital malformations, disruptions of spermatogenesis) supports the hypothesis that it might represent a form of manifestation of the testicular dysgenesis syndrome (TDS).

Macroscopic lesions are not evident. In optical microscopy, ITGCN cells appear similar to normal spermatogonia, but larger, with hyperchromatic nuclei, prominent nucleoli and abundant, clear, vacuolated cytoplasm. Relatively frequent mitoses (normal and abnormal) may be present, and spermatogenesis is usually much reduced/ absent. ITGCN cells are frequently arranged in a single layer located near the basal membrane of seminiferous tube and the tube wall is usually atrophic, sometimes thickened. Leydig and Sertoli cells are normal. Isolated malignant cells may be found in the rete testis and even the epididymis; if found in the interstitium or the lymphatic vessels, they are a marker of microinvasive disease (intermediate stage of evolution towards a GCT). Common stains are sufficient for diagnosis; when in doubt, PAS staining reveals large amounts of intracytoplasmic glycogen. In immunohistochemistry, placental alkaline phosphatase (PLAP) was highlighted as a practical, highly sensitive (83-99%) membrane marker of these cells. Others, such as ferritin, AFP, βHCG, EAA, CD117, M2A, TRA-1-60 are rarely and inconsistently positive. In electron microscopy, ITGCN are very similar to prespermatogonial cells in early differentiation stages. Genetic analysis shows that polyploidization of ITGCN DNA (hypotriploid/pentaploid) precedes the occurrence of i(12p); in terms of karyotype evolution, ITGCN is just one step behind invasive GCTs. Other changes are loss (chromosomes 4 and 13) and gain (chromosome 2p) of genetic material. Absence of chromosome i(12p) in childhood GCTs suggests that the pathogenesis may be different in this age group. The importance of ITGCN is pathogenic, diagnostic and prognostic. Approximately 50% of cases will progress to an invasive GCT in 5 years, and 90% in 7 years; in rare cases, ITGCN characteristics may remain unaltered [Sesterhenn and Davis, 2004].

Unlike most solid cancers, serum tumor markers play an extremely important role in both the positive and differential diagnosis of GCTs, as well as in their staging, prognosis, and follow-up. Some GCTs secrete LDH, AFP and βHCG, which are considered key serum indicators of the existence of testicular cancer, but not ideal tumor markers (virtually all seminomas and about 20% of teratomas do not produce them, and none are specific to testicular cancer). Still, seminoma patients may express slightly elevated βHCG (≤200μg/ml, 15% of cases) and PLAP levels (40% membrane, but no cytoplasmic positivity, unlike non-small cell lung cancer and malignant melanoma), and teratomas are, rarely, associated with high levels of AFP. Carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), antigen B5, nuclear epitope OCT3/4 (POU5F) and neuron-specific enolase (NSE) are still only of experimental interest [Bosl *et al.*, 2008].

Tumor markers may also help in the differential diagnosis of a metastatic EC (CAM5.2+, PLAP±, CD30±, EMA–) from a carcinoma (epithelial cancer) (CAM5.2+, PLAP–, CD30–, EMA+). Embryonal carcinomas are highly positive for various cytokeratins and OCT3/4, but variably for c-kit. Seminomas are generally CAM5.2–, CD30– and EMA–, but may have foci of positivity for these markers, and also for CK7 and AE1/AE3. Choriocarcinomas are βHCG-positive (syncytiotrophoblastic strand), and CK7-positive (cytotrophoblastic strand), and may also positively stain for HPL, EMA, other cytokeratins, inhibin, and PLAP (Table II). In some cases, it is accepted that apparently aberrant tissue expression of tumor markers does not alter the diagnosis or prognosis (e.g., seminoma with βHCG-positive syncytiotrophoblastic cells [10-20% of cases] or EC with increased serum βHCG [5% of cases]).

Table II. *Immunohistochemical markers of germinal testicular tumors* [Allen, 2006]

Histology	**AFP**	**βHCG**	**PLAP**	**CAM5.2**	**OCT3/4**	**CD117**	**CD30**	**EMA**
CIS / ITGCN	–	–	+	±	+	+		
Seminoma	±	±	+	–	+	+	–	–
Spermatocytic seminoma	–	–	–	–				
Yolk sac tumor (YST)	+	±	±	+				
Embryonal carcinoma (MTU)	±	±	±	+	+	±	±	–
Choriocarcinoma (MTT)	–	+	±	±				±

CONCLUSIONS

Considering the genomic instability and selection processes taking place during oncogenesis, the suggestion that testis tumors develop as a continuum is unusual, but is supported by the remarkable consistency of individual gene expression profiles of seminoma and non-seminoma. Testicular cancer has become a paradigm for the multimodal treatment of malignancies. It is also one of the few neoplasms associated with accurate serum markers, making it biologically unique among solid tumors. Adoption of immunohistochemistry, cytogenetic analysis of chromosomal abnormalities, and other high throughput techniques such as identification of new biologically significant substances (e.g., proteins from oncogene expression by proteomics) – has been giving new meaning to the ‘tumor marker’ notion.

Yet, there is still a need to accurately identify poor prognosis patients, who might benefit from more aggressive treatment strategies. Because there is still no animal model available to represent the characteristics of human GCTs, studies carried out on testicular stem cells in culture or in an animal host will bear great significance for understanding the pathogenic mechanisms of this particular neoplasia, and possibly find ways of extending their exquisite chemosensitivity to other malignant tumors.

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