Communication

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An up-date on novel molecular targets in testicular germ cell tumors subtypes

Paolo Chieffi*

Dipartimento di Psicologia, Università della Campania, Caserta, Italy.

Summary **Testicular germ cell tumors (TGCTs) are the most frequent solid malignant tumors in men 20-34 years of age and the most frequent cause of death from solid tumors in this age group. In addition, the incidence of these tumors has significantly increased over the last few decades. Testicular germ cell tumors are classified into seminoma and nonseminoma germ cell tumors (NSGCTs). NSGCTs can be further divided into embryonal carcinoma, Teratoma, yolk sac tumor, and choriocarcinoma. There are noteworthy differences about therapy and prognosis of seminomas and nonseminoma germ cell tumors, even though both share characteristics of the primordial germ cells (PGCs). Many discovered biomarkers including HMGA1, GPR30, Aurora-B, estrogen receptor β, and others have given further advantage to discriminate between histological subgroups and could represent useful molecular therapeutic targets.**

Keywords: Testicular germ cells tumors, seminomas, Aurora B, GPR30, PATZ1, HMGA

Testicular germ cell tumors (TGCTs) have the highest incidence among young men (between 20 and 34 years of age) of solid tumors, and their incidence has significantly increased over the last few decades. About 90% of TGCTs are successfully treated with cisplastin-based chemotherapy. However, this kind of therapy raises the possibility of developing secondary cancers and cardiovascular disease. TGCTs are classified into two principal groups: Germ Cell Neoplasias *In Situ* (GCNIS) that are Seminoma and Nonseminoma (NSE), and spermatocytic tumors that are not GCNIS. NSE tumors encompass embryonal carcinoma, choriocarcinoma, Yolk Sac Tumors (YSTs) and teratomas. TGCTs may develop from a non-invasive type of tumor called carcinoma *in situ* (CIS): Microscope analysis reveals abnormal cells even though they are still confined inside the membrane of the seminiferous tubules (*1-9*).

A significant increase in TGCTs incidence occurred in the last few decades, probably due to altered environmental factors that significantly contribute to disease onset. For instance, although the biological mechanisms are still unclear, evidence suggests that the risk of developing TGCTs is associated with maternal smoking during pregnancy, adult height, biomass index, diet rich in cheese, pesticide exposure, and others. Among the risk factors involved in the onset of disease: age, cryptorchidism, family history of testicular cancer, Klinefelter's syndrome, personal history of testicular cancer, congenital abnormalities and infertility. Cryptorchidism is the major risk factor associated with germ cell tumors: it deals with undescended testicle into the scrotum, which remains in the abdomen or groin, thus the risk of developing the disease does not change even after surgery to move the testicle into the scrotum. Remarkably, it is still debatable whether the exposure to some nonsteroidal estrogens during pregnancy, such as diethylstilbestrol (DES) may increase the risk of developing TGCTs. Despite that this divergent evidence confirms the important role played by some environmental factors in TGCTs, etiology has been clearly suggested by migration studies. Consistently, Sweden has an incidence of TGCTs about twice that of Finland and, although first generation migrants from Finland to Sweden show no increased risk, second generation males born to the migrant parents in Sweden present an increased frequency (*10,11*).

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**Address correspondence to:*
Dr. Paolo Chieffi, Dipartimento di Psicologia, Università della Campania, Viale Ellittico, 3181100 Caserta, Italy.
E-mail: paolo.chieffi@unicampania.it

Numerous new biomarkers have been found to discriminate TGCTs subtypes, standing for innovative molecular therapeutic targets. High-mobility group proteins A1 (HMGA1) and A2 (HMGA2) act as powerful diagnostic markers (*12-15*). Really, these two proteins are diversely expressed in TGCTs in comparison with the stage of tumor differentiation (*12,13*). For example, HMGA1 binds to other proteins, such as RNF4 (*16,17*) and PATZ1, which are engaged in transcriptional control and have been demonstrated to be overexpressed are delocalized in human testicular seminomas (*18*). Currently, we have shown that in human testicular seminomas Estrogen Receptor β (ERβ) expression is strongly down regulated and this down regulation is associated with delocalization of both PATZ1 and HMGA1 transcriptional factors, on the contrary, in normal germ cells, PATZ1 binds to ERβ (*19,20*).

The serine/threonine kinase NEK2 is a key regulator of centrosome separation and bipolar spindle formation during mitosis and chromatin condensation during meiosis. It controls centrosome separation (essential for the formation of bipolar spindles and high-fidelity chromosome separation) through the phosphorylation of proteins such as CEP250, CROCC and NINL, causing their dislocation from the centrosomes. Additionally, NEK2 has a major function in chromatin condensation in the first meiotic division by HMGA2 phosphorylation (*21*). Moreover, the enhancement and the nuclear localization of NEK2 protein has been found in both seminomas and in seminoma cell line (TCam-2) (*22,23*). Furthermore, recent studies underlined the new splicing factor kinase function of NEK2 (*23*).

The RNA-binding protein LIN28 is implicated in the maintenance of the pluripotency of embryonic stem cells, and its expression levels are reduced throughout differentiation. In particular, LIN28 regulates the expression of OCT4 through directly binding to its mRNA transcript in mouse embryonic stem cells. Indeed, LIN28 has a pivotal function for reprogramming somatic cells into pluripotent stem cells. Moreover, LIN28 represents a valid diagnostic marker for testicular GCNIS, classical seminomas, embryonal carcinomas, and YSTs (*24*). In particular, LIN28 is the main YST marker due to the absence of OCT4 (*24*).

Estrogen signaling is mediated by two nuclear receptors, estrogen receptor α (ERα) and β (ERβ), that are estrogen dependent transcription factors. ERα is expressed at high levels in human epididymis and efferent ductules, but not in the testis, whereas ERβ is expressed in spermatogonia, spermatocytes, and in early round spermatids in human testis (*1,2*). The ERβ subtype is the principal mediator of estrogen action in promoting germ cell survival and development. After activation, these receptors, in association with a myriad of co-activators and repressors, act as nuclear transcription factors for targeted genes. It has been well documented in the literature that ERβ, which is expressed in normal testicular cells, is instead down regulated in seminomas and embryonal cell carcinomas (*1,2*). Until recently, the estrogen receptors α (ERα) and estrogen receptors β (ERβ) (*25-27*) have been considered the major physiologic estrogen mediators. Indeed, the G protein-coupled estrogen receptor (GPR30) has proved to have an increasing role in estrogen-mediated signalling in a wide variety of cell types. The critical role of GPR30 in preservation and in development and homeostasis of normal testis is well recognized (*28-30*). Recent studies show that GPR30 is overexpressed in human spermatogonia, spermatocytes (*29*), in the TCam-2 cell line and seminomas. Moreover, it has been verified that ERβ downregulation correlates with GPR30 overexpression both in human CIS and seminomas; furthermore, it has been demonstrated that 17β-estradiol produces ERK1/2 activation through GPR30 (*31*). Many studies are committed to develop novel therapeutic strategies for the treatment of TGCTs blocking neoplastic germ cells through the design of selective GPR30 inhibitors.

The kinase Aurora-B is another valuable marker able to discriminate among the different tumor histotypes; in fact, it is detected in IGCNU, seminomas and embryonal carcinomas, but not in teratomas and YST. Pharmacological inhibition of Aurora B significantly decreases the cell growth in testicular GC1 and TCam2 cell lines (*32-35*).

Perturbation of miRNAs plays an important role in the establishment and progression of many cancer types, including TGCTs (*36*). Although different miRNA signatures are associated with histological subtypes of TGCT, very few miRNAs have been found to have a key role in TGCTs. Indeed, Dicer knockout mice show a premature reduction of germ cell numbers and deregulated differentiation of male germ cells (*36*). Then, Voorhoeve *et al*. showed that miR-372 and miR-373 may overcome p53-mediated arrest of the cell cycle (*37*). Conversely, miR-372 and miR-373 were absent in TGCT-derived cell lines with mutated p53 or expressed low levels of p53, suggesting that these miRNAs may allow the growth of TGCT escaping the p53 checkpoint of the cell-cycle. In this context, data suggests that miR-372 and miR-373 may act as oncogenes in TGCT through the inhibition of LATS2, a tumor suppressor gene (*36*). Moreover, the novel identification of circulating miRNAs in body fluids like serum, may represent a valid non-invasive manner to diagnosis and follow disease status. In this regard, it has been reported that miR-371 and miR-372 are specifically increased in serum of germ cell tumor patients. Moreover, many other miRNAs have been proposed to be able to discriminate between different tumor hystotypes, confirming the function of the embryonic miR-371 and miR-372 in identifying malignant TGCT (*38*).

Pseudogenes have long been considered as non-

functional genomic sequences. However, recent evidence suggests that many of them might have some form of biological activity, and the possibility of functionality through a microRNA-mediated pathway (*39*). Recently, two HMGA1 processed pseudogenes (HMGA1P6 and HMGA1P7) were isolated. In particular, these pseudogenes, competing with HMGA1 for microRNA binding, lead to the upregulation of HMGA1 cellular levels, exerting an oncogenic role (*40*). In this context, although further experiments are needed, preliminary data show that HMGA1 pseudogenes are differentially overexpressed in TGCT histotypes in comparison with normal testis (seminomas, embryonal carcinomas, mixed form teratomas, and YSTs), suggesting a role of HMGA1 pseudogenes in TGCT carcinogenesis.

The development of human TGCTs is subjected to genetic and environmental factors that have a crucial role in deregulating the normal differentiation process in PGCs. Recently, the increasing number of tumor biomarkers has permitted histological discrimination among the various subgroups. A better comprehension of the molecular pathways through which the TGCTs develop will point out new tools to definitely target cancer cells and will help to defeat intrinsic and acquired chemotherapy resistance. Aurora-B serine-threonine kinases, HMGAs and GPR30 inhibitors (*41,42*) are promising molecules able to selectively target cancer cells, introducing a new scenario for TGCTs treatment in the near future.

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