Communication

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An up-date on epigenetic and molecular markers in testicular germ cell tumors

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Summary **Testicular germ cell tumor (TGCT) is the most common solid malignancy occurring in young men between 20 and 34 years of age, and its incidence has increased significantly over the last decades. Clinically several types of immunohistochemical markers are useful and sensitive. These new biomarkers are genes expressed in primordial germ cells/gonocytes and embryonic pluripotency-related cells but not in normal adult germ cells and they include OCT3/4, HMGA1 and 2, NANOG, SOX2, and LIN28. Gene expression in TGCT is regulated, at least in part, by DNA and histone modifications, and the epigenetic profile of these tumours is characterised by genome-wide demethylation. There are different epigenetic modifications in TGCT subtypes that reflect the normal developmental switch in primordial germ cells from an under to normally methylated genome.**

Keywords: Testicular germ cells tumors, seminomas, epigenetic, GPR30, PATZ1, HMGA

Testicular germ cell tumor (TGCT) is the most common neoplasia that occurs in males between 20-40 years old and it accounts for approximately 1-1.5% of all cancers in men (*1,2*). The incidence rate and mortality change considerably in different geographical areas: the rates are highest in Northern and Western Europe, Northern America and Australia, while lowest rates have been found in South Europe, Central America and, at last, in Asia and Africa (*3*). Over the last decades, the incidence of TC in western countries has been increasing, maybe because of an increased exposure to etiologic factors (*4*). Genetic and environmental factors play an important role in the genesis and development of TGCT; in fact, several genes are implicated in its pathogenesis (*5*) and different environmental factors have been investigated. In the environmental agents there are pesticides and non-steroidal estrogens, such as diethylstilbestrol (DES) (*6,7*). There is an association between increased TC risk and maternal smoking during pregnancy, adult height, body mass index, diet rich in cheese are other factors correlated to TGCT development (*8*). However, the biological mechanisms involved in TGCT development are poorly known. Among the risk factors correlated to the onset of disease we can remember: age, cryptorchidism, family history of TGCT, Klinefelter's syndrome, congenital abnormalities and infertility (*2,3*). Young age represents one of the most frequent factors of TGCT occurrence (*3*).

The World Health Organization (WHO) recapitulates the classical histological entities of testicular cancers, but now divides the tumours of adult men into two main groups: those that are derived from germ cell neoplasia *in situ* (GCNIS): seminoma and nonseminoma (NSE), and a spermatocytic tumor, which is a histotype not associated with GCNIS. In NSE are included choriocarcinoma, embryonal carcinoma, teratoma and yolk sac tumors. Testicular germ cell tumors may arise from a non-invasive form of disease named carcinoma *in situ* (CIS): under the microscope these cells appear abnormal although they have not yet spread outside the walls of the seminiferous tubules. CIS doesn't always degenerate in invasive cancer but it's very difficult to discover it because it often doesn't involve side effects; a good way to diagnose CIS is to have a biopsy. According to its evolution, three stages of the disease can be distinguished: stage I (the tumor is circumscribed by the testicle), stage II (the tumor has spread to the lymph nodes of the abdomen) and stage III (the tumor has spread to the lymph nodes also with distant metastases in organs such as lungs and liver) (*9*).

Compared to the latest classification of urinary tract

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and male genital organs in the WHO, there have been updates in 2016 that reflect the different behaviour, pathogenesis and tumour biology of similar histological patterns occurring in different contexts. In particular, GCNIS has been used as a new name for the precursor lesion (*9*). TGCT is a developmental disease of germ cell differentiation, and almost all TGCTs are derived from dysfunctional fetal germ cells. The characterization of gene expression profile in seminoma may be useful not only to improve our knowledge on their relation with oncogenesis, but also to better understand the role of PGCs (*10-13*).

NSE tumors are usually treated with surgery and chemotherapy, with different cure rates depending on the stage of the disease. The cure rate reaches up to 99% in the early stages of NSE tumors, although in advanced disease stages decreases from 90% in patients with good prognostic category to 50% in patients with poor prognostic features (*10-13*).

The fast progression and the rapid growth of postpuberal TGCTs cause early lymph node metastases and/or distant metastases. In fact, about 25% of patients with seminoma and up to 60% of those with NSE suffer from metastatic disease (*10-13*), and the therapeutic treatments are often ineffective. Thus, despite the general success of postpuberal TGCTs treatment, 10-20% of patients diagnosed with metastatic disease will not achieve a durable and complete remission after initial treatment, either due to incomplete response or a tumor relapse.

There are several types of markers for testicular TGCTs: serum tumor markers and immunohistochemical markers. Clinically useful immunohistochemical markers for TGCT are genes expressed in primordial germ cells (PGCs)/gonocytes and embryonic pluripotency-related cells but not in normal adult germ cells. They are OCT3/4, NANOG, SOX2, REX1, AP-2γ (TFAP2C) and LIN28. Moreover, HMGA1 and HMGA2 represent valuable diagnostic markers as they are differently expressed depending upon the states of differentiation of TGCTs (*15-25*). A nuclear transcriptional repressor PATZ1 interacting protein RNF4 suggests an impaired function when it is delocalized in the cytoplasm in human seminomas; it has been shown that both PATZ1 and HMGA1 cytoplasmic delocalization associates with estrogen receptor β (ER β) down-regulation in human seminomas (*15-24*). More recently, it has been demonstrated that the down regulation of ERβ associates with GPR30 over-expression both in human CIS and seminomas; in addition, it has been shown that 17β-estradiol induces the ERK1/2 activation through GPR30 (*25,26*). Many studies are devoted to design selective GPR30 inhibitors to block neoplastic germ cells with a high proliferative rate, representing a novel therapeutic strategy for the treatment of TGCTs (*25,26*).

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 (*26-29*).

During recent years the epigenetic factors have been found to be extremely important in the development of cancer. Indeed, nuclear morphologies are often pleiotropic across a single tumor, reflecting the heterogeneous nature of cancer. These modifications and changes of nuclear structure are key features distinguishing cancer cells from their normal counterparts. Altered nuclear morphology also reflects broad changes in genome positioning and epigenetic changes, which occur during transformation. In particular, in cancer the tight regulation of DNA methylation and the distribution of methyl-cytosine change. Commonly, the heavy methylation in the bulk chromatin is reduced, while the normally unmethylated CpG islands become hypermethylated (*30,31*). In testicular GCTs it is important to evaluate DNA methylation in the context of the PGCs from which the tumors arise because PGCs are at a developmental stage where their genomes are highly under-methylated (*30,31*). Several studies have demonstrated minimal or no methylation in seminomas, and hypermethylation in specific gene promoters of NSE, especially in highly differentiated NSE, suggesting that the degree of cell differentiation may be related to the genome methylation status (*30,31*). CIS cells as PGCs and gonocytes, express transcription factors associated with embryonic stem cell pluripotency, such as POU5F1/OCT-3/4, NANOG, T1A-2, MYCL1, GDF3, LIN28-A, DPPA4, DPPA5, KIT and AP-2γ. Genome-wide gene expression profiling revealed specific embryonic stem cell-like features of testicular CIS (*30-32*). The epigenetic pattern of CIS is associated with an open and permissive chromatin structure based on expression pattern of genes and transcription factors.

A new dawn is arising in the scenario of TGCT diagnostic and prognostic classification based upon the use of epigenetic molecular markers.

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