Potential New Anticancer Molecular Targets for the Treatment of Human Testicular Seminomas

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Abstract: In the last years novel therapeutic approaches for the treatment of cancer have been proposed: specific inhibitors of serine/threonine and tyrosine kinases, angiogenesis inhibitors, antibodies against receptors/surface molecules on cancer cells, gene therapy approaches and others. In a lot of cases the clinical trials have confirmed the efficacy of these approaches.

Here, we will review the discovered new potential molecular targets for the treatment of human testicular seminomas.

Keywords: Testis, testicular cancer, gonocytes, testicular germ cells, seminomas.

INTRODUCTION

Although testicular germ cell tumors (TGCTs) are relatively uncommon, they are particularly important as they tend to affect children and young men, representing the most common tumor in male aged from 20 to 40 years.

TGCTs of the testis are a heterogeneous group of neoplasms. They are classified as seminomatous (SE-TGCT) and nonseminomatous (NSE-TGCT) tumors [1-3]. Distinction of prebuberal TGCTs, exclusively represented by yolk sac tumor (YST) and teratoma, and postpuberal TGCTs, invariably arising from intratubular germ cell neoplasias (ITGCNs) seems to have a great prognostic relevance [4,5].

Postpuberal TCGTs are the most frequent solid malignant tumors in men between 20 and 40 years of age, accounting for up to 60% of all malignancies diagnosed at this age. Despite a high-cure rate, they represent the most frequent cause of death from solid tumors in this age group [1-3]. Seminomas are radio- and chemo-sensitive tumors [6]. NSE tumors are usually treated with surgery and chemotherapy, with different cure rates depending on the disease stage [7]. The cure rate reaches up to 99% in the early stages of NSE tumors, although in advanced disease decreases from 90% in patients with good prognostic category to 50% in patients with poor prognostic features [7].

The rapid growth and progression of postpuberal TGCTs cause early lymph node metastases and/or distant metastases. At the time of diagnosis about 25% of seminoma patients and up to 60% of the nonseminoma patients suffers from metastatic disease [8-10], posing a therapeutic problem since in metastastic disease the treatment achieves modest results. Thus, despite the general success of postpuberal TGCTs treatment, 10–20% of patients diagnosed with metastatic disease will not achieve a durable complete remission after initial treatment, either due to incomplete response or a tumors relapse. TGCTs show a high-cure rates in both seminomas and nonseminomas and represent the model of a curable neoplasia: sensitive serum tumor markers, accurate prognostic classification, contribute to a high effectiveness of cancer therapy.

These different prognostic and therapeutic features of TGCTs highlight the need for a better understanding of the molecular biology of TGCTs, that could help to improve disease management and to tailor aggressiveness of treatment to the severity of the prognosis.

The review will focus on the molecular alterations identified in postpuberal human testicular seminomas and on novel targeted molecular antineoplastic strategies.

EPIDEMIOLOGY AND RISK FACTORS

TGCTs have significantly increased in the past 50 years; this increase is probably due to changes in environmental factors contributing to the development of these lesions. A number of environmental factors have been investigated to explain the possible links. Some evidence suggests association of increased TGCTs risk and maternal smoking during pregnancy, adult height, body mass index, diet rich in cheese, and others [11-15], however, the biological mechanisms remain to be elucidated.

Hypothesized environmental agents involved in the development of TGCTs, include pesticides [16] and nonsteroidal estrogens, such as diethylstilbestrol (DES) [17]. It has been proposed that increased levels of estrogen exposure *in utero* to increase the risk of TGCTs [18] and the exposure of women to the nonsteroidal estrogen DES during pregnancy increases the risk of TGCTs [19]. However, other studies have not confirmed a role for estrogen in TGCTs development [20]. Despite the contrasting results reported in the literature a clear role for environmental factors in the etiology of TGCTs is suggested by population migration studies. Sweden has an incidence of TGCTs about twice that of Finland and although first generation migrants from Finland to Sweden show no increased risk [21], second

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generation males born to the migrant parents in Sweden have a tendency to an increased frequency [22].

Familial predisposition to TGCTs, ethnic variations in incidence, and an association with certain chromosome abnormality syndromes strongly suggest that inherited factors, also, play a role in disease development. The familial predisposition is one of the strongest for any tumor type, since the increased relative risk of TGCTs development associated with fathers and sons of TGCTs patients is fourfold [23]. However, gene(s) involved in familial TGCTs have not been identified so far [3]. Genome-wide linkage analysis of affected families has provided evidence for two susceptibility loci, one at Xq27 locus for undescended testis probably playing an indirect role and another at 12q which results in hyperexpression of the product of the CCND2 gene [24]. It is probable that both genetic and environmental factors produce the high familial risk seen in TGCTs and that the interplay between these two factors, along with genetic heterogeneity, may make familial associated susceptibility loci difficult to determine.

HISTOPATHOLOGY

The origin and biology of TGCTs are currently distinct on whether they occur in pre- and postpuberal age, being pure teratomas and YSTs with a substantially benign prognosis the most common hystotypes of prepuberal testis and seminoma, pure NSE tumors and mixed germ cell tumors (GCTs) with a relative more aggressive behavior typical of adult testis [2,3].

It has been suggested that the initiating event in the pathogenesis of TGCT occurs during embryonal development [1,2]. The most widely accepted model of postpuberal TGCTs development proposes an initial tumorigenic event *in utero* and the development of a precursor lesion known as intratubular germcell neoplasia undifferentiated (ITGCNU), also known as carcinoma in situ (CIS) [25]. This is followed by a period of dormancy until after puberty when postpuberal TGCTs emerge. This prepubertal dormancy suggests that the TGCTs development is hormone dependent.

Recently, it has been proposed that tumors originate from neoplastic cells that retain stem cell properties such as self-renewal [26], and this novel hypothesis has fundamental implications for the pathogenesis of TGCTs. According with stem cells hypothesis, tumors originate from tissue stem cells or from their immediate progeny. This cellular subcomponent drives tumorigenesis and aberrant differentiation, contributing to cellular heterogeneity of the tumor and also to the resistance to antineoplastic treatments.

ITGCNU cells are generally accepted as the common preinvasive precursor cells that gives rise to postpuberal TGCTs [3,27]. ITGCNU almost found invariably in the periphery of overt postpuberal TGCTs and is estimated that it is present in approximately 5% of the contralateral testis of patients with postpuberal TGCTs [28]. Preinvasive ITGCNUcells are supposed to be able to develop in different germinal and somatic tissues and are regarded as pluripotent or totipotent cells and therefore can be considered as TGCTs stem cells. ITGCNU cells share morphological similarities with gonocytes and it has been proposed that ITGCNU cells could be remnants of undifferentiated embryonic/fetal cells [29,30].

Their fetal origin is also supported by immunohistochemical studies of proteins present in ITGCNU, also shown to be present in primordial germ cells (PGCs) and gonocytes. The identification of ITGCNU cells in prepubertal patients, who later developed TGCTs, indicated that the cells had originated prior to puberty [31].

Therefore, ITGCNU cell represents an interesting variant of cancer stem cell since it originates before the tissue that it propagates in is fully differentiated and functional. The observation that two transcription factors, POU5F1 (OCT3/4) and NANOG, known to be associated with pluripotency in ES cells are expressed in ITGCNU has further contributed to assess the embryonic origin of these cells. A link between ITGCNU cells and embryonic cells has been further supported by a substantial overlap between human ES cells and ITGCNU cells gene expression profiles, as shown by Almstrup *et al.* [32]. All hystotypes could be present in postpuberal TGCTs, because of its totipontent profile, even seminoma can switch to nonseminoma hystotype through reprogramming phenomenon (Fig. (**1**)) [33-35]. The role of these factors will be discussed in more detail in the next sections.

Seminoma consists of transformed germ cells, that closely resemble the PGC/gonocyte, apparently blocked in their differentiation. Nonseminoma could be constituted by cells with typical pluripotency of PGC/gonocyte. In particular, embryonal carcinoma reflect undifferentiated stem cells, Teratoma represent somatic differentiation, while choriocarcinoma and YST extraembryonal differentiation. Genetic studies have shown that postpubertal testis tumors are often aneuploid with a consistent chromosomal abnormality composed of a gain of short arm of chromosome 12, usually in the form of an isochromosome, i(12p). In contrast tumors arising in prepubertal gonads are typically unassociated with 12p amplification and tend to be diploid. The most consistent structural chromosomal abnormality is an isochromosome 12p. Tumors lacking i(12p) have other structural abnormalities of 12p, among them the amplification of 12p11.2–p12.1. Gain of 12p sequences may be related to invasive growth [5] suggested that cyclin D2 (mapped to 12p13) is the most likely candidate gene of pathogenetic relevance.

NEW DISCOVERED MARKERS AS POTENTIAL TARGETS OF HUMAN SEMINOMAS

A number of markers has been reported over time that can be used to discriminate CIS, seminoma, embryonal carcinoma, teratoma, and yolk sac and they could be sed as potential molecular therapeutic targets. The most common are HMGA1, HMGA2, PATZ1, Aurora-B, Nek2, OCT3/4, c-Kit, PLAP, NANOG, SOX2, GPR30 and others. For example, HMGA1 and HMGA2 are differently expressed with respect to the state of differentiation of TGCTs, with overexpression of both proteins in pluripotential embryonal carcinoma cells and loss of expression of HMGA1 in YSTs and of both proteins in mature adult tissue of teratoma areas.

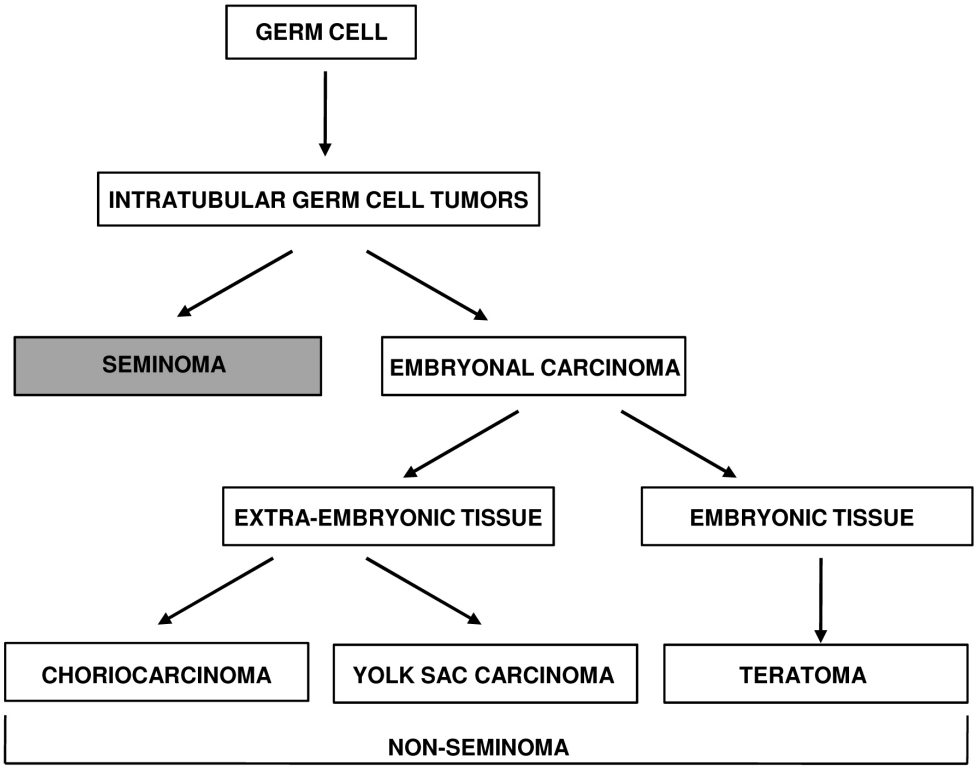

Fig. (1). A scheme illustrating current understanding of the pathogenesis of Testicular Germ Cell Tumors.

Therefore, the different profile of HMGA1 and HMGA2 protein expression could represent a valuable diagnostic tool in some cases of problematic histological differential diagnosis [36,37].

PATZ1 is a recently discovered zinc finger protein that acts as a transcriptional repressor. Although expression of PATZ1 protein was increased in TGCTs, it was delocalized in the cytoplasm, suggesting an impaired function [38]. More recently it has been shown that PATZ1 cytoplasmic delocalization associates with estrogen receptor β (ERβ) down-regulation in human seminomas [39]. Another marker that could be used to discriminate the different tumor histotype is Aurora-B expression; in fact, it was detected in all CIS, seminomas and embryonal carcinomas analyzed but not in teratomas and yolk sac carcinomas [40,41]. Aurora-B will be discussed in more detail later.

It is shown that Nek2 protein, a centrosomal kinase required for centrosome disjunction and formation of the mitotic spindle, is upregulated and localized in the nucleus of neoplastic cells of seminomas. Such nuclear localization and the upregulation of Nek2 protein were also observed in the Tcam-2 seminoma cell line. In addition, the nuclear localization of Nek2 is a feature of the more undifferentiated germ cells of mouse testis and correlates with expression of the stemness markers OCT4 and PLZF [42]. OCT3/4 is a well-characterized marker for PGCs. It is positive in all cases of CIS, seminoma, and embryonal carcinoma[43,44]. There has been a various amount of reports over the years that OCT3/4 is also expressed in normal adult stem cells and nongerm cell-derived cancers. However, recent data indicate that these observations are likely related to the use of nonspecific antibodies, the latter also recognizing pseudogenes [45-48]. OCT3/4 is a transcription factor of the family of octamer-binding proteins (also known as the POU homeodomain proteins) and is regarded as one of the key regulators of pluripotency [49]. In addition to OCT3/4, several other embryonic stem-cell-specific proteins are important for maintaining the so-called ‘‘stemness’’ of pluripotent cells, such as NANOG and SOX2 [50-53].

NANOG protein was detected in germline stem cells (gonocytes) within the developing testis. In addition, NANOG is highly and specifically expressed in CIS, embryonal carcinoma, and seminomas, but not in teratoma and YSTs revealing a molecular and developmental link between GCTs and the embryonic cells from which they arise [54].

SOX2 is a member of the SOX protein family, transcription factors that regulate development from the early embryonal stage to differentiated lineages of specialized cells. SOX proteins are known to cooperate with POU proteins. The best characterized SOX–POU cooperation is that between SOX2 and OCT3/4. SOX2 is not detected in human germ cells regardless of their developmental age, in contrast to data in mouse embryos [55]. SOX2 is expressed in embryonal carcinoma, the undifferentiated part of nonseminomas, but it is absent in seminomas, YSTs, and normal spermatogenesis [55]. CIS cells are indeed negative for SOX2, although SOX2 positive Sertoli cells can be present in seminiferous tubules lacking germ cells or in the presence of CIS [55].

Expression analysis of SOX family members in TGCTs revealed that is specifically expressed in CIS and seminoma

but not in embryonal carcinoma [55]. In addition, SOX17 maps to the chromosomal region 8p23, which is gained in seminoma [56]. This indicates that SOX17 is a candidate SOX protein for cooperation with OCT3/4 in CIS and seminoma. These data also illustrate that SOX17 is a new marker to discriminate CIS and seminoma from embryonal carcinoma. Of interest is that SOX17 distinguishes embryonic from adult hematopoietic stem cells [57]. Current research focuses on the processes that may regulate the differential expression of SOX2 versus SOX17 and on the role of these SOX proteins in the different histologies of the TGCT subtypes involved. Analysis of expression patterns in microarray studies revealed additional markers, MCFD2, BOB1, and PROM1, for seminoma compared to normal testis [58]. Studies demonstrated indeed increased expression levels of these three proteins in seminoma cells compared to normal adult testes [59]. Because all three of these markers are also expressed at low levels in normal adult testicular tissue, their suitability as practical additional diagnostic markers remains to be proven.

Although the physiologic responses to estrogens are mainly mediated by the ERα and ERβ [60-62], in the last few years, GPR30 has been shown to mediate estrogen signaling in a wide variety of cell types. GPR30 is an intracellular 7-transmembrane G protein-coupled estrogen receptor (GPR30) that functions alongside the traditional estrogen receptors (ERα and ERβ) to regulate physiological responsiveness to estrogen. It has been shown that GPR30 is overexpressed in seminomas and in the derived human seminoma TCam-2 cell line indicating that it could be a good potential therapeutic target; [63,64].

In recent years, the role of miRNAs in carcinogenesis of human testicular cancer and germ cell development has emerged [65]. It was demonstrated that knockout mice for Dicer suffered from an early decrease in germ cell number and an impaired ability to differentiate, indicating that Dicer1 and miRNAs are important for both survival and proper differentiation of male germ cells [65]. Subsequently, it was demonstrated that miRNAs 372 and 373 can overcome cellcycle arrest mediated by p53 [66]. In contrast, in TGCT cell lines with mutated p53 or expressing low levels of p53 were shown to be negative for these miRNAs and it can be assumed that miRNAs 372 and 373 can bypass the p53 checkpoint allowing the growth of TGCT. Further research into the functional mechanisms of miRNAs and the role of DND in TGCT are likely to give more interesting clues.

AURORA KINASE INHIBITORS

Errors in mitosis can provide a source of the genomic instability that is typically associated with tumorigenesis. Many mitotic regulators are aberrantly expressed in tumor cells. The kinases Aurora-A, -B, and -C represent a family of protein well conserved throughout eukaryotic evolution and members of this family have been extensively studied in a range of different model organisms [67,68]. All three mammalian members of this family are overexpressed in human cancer cells [67]. Although the catalytic domains of the Auroras are highly conserved, these proteins show different subcellular localizations. Aurora-A (STK-15) localizes to the duplicated centrosomes and to the spindle poles in mitosis. It has been implicated in several processes required for building a bipolar spindle apparatus, including centrosome maturation and separation. Aurora-A has been found to be overexpressed in the meiotic testicular cells [69]. It is interesting to note the aneuploidy of human TGCTs is associated with amplification of centrosomes [69]. Aurora-B (AIM-1) is a chromosomal passenger protein. Aurora-B binds three other chromosome passenger proteins-innercentromere protein (INCENP), survivin, and borealin [67,68]. During mitosis, Aurora-B is required for phosphorylation of histone H3 on serine 10, and this might be important for chromosome condensation [67,68]. Aurora-B clearly regulates kinetochore function, as it is required for correct chromosome alignment and segregation. Aurora-B is also required for spindlecheckpoint function and cytokinesis [67,68].

Aurora-A and -B are overexpressed in primary breast and colon tumor samples [70]. Aurora-A is localized (20q13) to an amplicon associated with poor prognosis in patients with breast and colon tumors [70]. Many studies have identified other tumor types, in which Aurora-A was amplified or overexpressed [67]. Aurora-C(STK-13) is also overexpressed in colorectal cancers [67]. The distribution and the expression of Aurora-B were investigated in neoplasms derived from germ cells showing that the expression of Aurora-B is a consistent feature of human seminomas and embryonal carcinomas suggesting that Aurora-B is a potential target in the therapy of TGCTs [40,41]. The increase of Aurora B expression in TGCTs has been confirmed by using Ki67 and PCNA as molecular markers [40,41,71]. Three Aurora-kinase inhibitors have recently been described targeting the enzymatic activity of the Aurora kinase and in particular blocking Aurora-B activity: ZM447439, Hesperadin 8 and VX-680 [67,68]. AZD1152, is a reversible ATP-competitive Aurora inhibitor, AZD1152 is 1000-fold more selective for Aurora kinase B than for Aurora kinase A, being the Ki values of 0.36 versus 1300 nM, respectively [67,68]. AZD1152 has shown highly significant tumor growth inhibition in a diverse panel of solid human cancer tumor xenograft models, including lung and colorectal cancers and his good solubility makes it suitable for clinical use. AZD1152 and other Aurora inhibitor are currently in early clinical evaluation, showing reversible neutropenia as major side effect. All these molecules act by inhibiting phosphorylation of histone H3 on serine 10 and consequently blocking cell division [67,68]. Although germinal cell tumors are highly responsive to commonly used chemotherapeutic treatment, cases of acute toxicity and chronic collateral effects, such as sterility, are recorded. Therefore, the availability of novel drugs such as Aurora-B inhibitor(s) could represent an escape from chemotherapy early and late effects.

RECEPTOR AND NONRECEPTOR TYROSINE KINASE INHIBITORS

Protein phosphorylation plays key roles in many physiological processes and is often deregulated in neoplastic lesions. Current understanding of how protein kinases and phosphatases orchestrate the phosphorylation changes that control cellular functions, has made these

enzymes potential drug targets for the treatment of different types of cancer. Recently, receptor and nonreceptor tyrosine kinases (TKs) have emerged as clinically useful drug target molecules for treating cancer [72]. Imatinib mesilate (STI-571) was primarily designed to inhibit bcr-abl TK activity and to treat chronic myeloid leukemia. STI-571 is also an inhibitor of c-Kit receptor TK, and is currently the drug of choice for the therapy of metastatic gastrointestinal stromal tumors (GISTs), which frequently express constitutively activated forms of the c-Kit-receptor [68]. Platelet-derived growth factor receptor-a (PDGFRa), and c-Kit are expressed at high levels in TGCTs [73,74].

The c-Kit/stem cell factor system is a signaling pathway for migration and survival of PGCs [75]. c-Kit is a tyrosine kinase receptor for the stem cell factor, ligand binding leads to the c-Kit receptor heterodimerization and tyrosine kinase activity and the downstream signal involves both apoptosis and cell cycle progression [75]. Activating mutations of c-Kit have recently been found in 93% of bilateral TGCTs, albeit in less of 2% of unilateral TGCTs [76]. These mutations affect codon 816 of c-Kit gene resulting in a constitutional kinase active, in a manner similar to other receptorial tyrosine kinase activating mutations [76]. However, the mutation in exon 17 is not inhibited by the tyrosine kinase inhibitor imatinib mesylate [77].

The success of the tyrosine kinase inhibitors in the treatment of some cancers has invigorated the development of kinase inhibitors as anticancer drugs and a large number of these compounds are currently undergoing clinical trials and it is likely that molecules capable to inhibit exon 17 c-Kit activating mutations will be identified contributing to the development of molecular targeted therapies.

ANGIOGENESIS INHIBITORS

Tumors require access to blood vessels for the supply of oxygen and to maintain growth. The development and the growth of new vessels (angiogenesis) are essential for tumor growth and progression. Judah Folkman in the early 1970s proposed the inhibition of tumor blood vessel as a therapeutic approach for treating cancer patients [78]. The blood vessel growth in normal tissues is regulated through a balance between the action of proangiogenic factors, such as vascular endothelial growth factor (i.e., VEGF) [79] and the action of angiogenic inhibitors (i.e., thrombospondin-1) [80]. In neoplastic lesions the angiogenic balance is shifted toward the proangiogenic factors, and irregular and uncoordinated tumor vessel growth is the result.

VEGFR tyrosine kinase, p53, cyclooxygenase-2 (COX-2), andmatrixmetalloproteinases (MMPs) all directly and/or indirectly influence the proangiogenic switch [79]. More than five inhibitors of the VEGF pathway have entered clinical phases I–III trials. Bevacizumab (Avastin(TM)), an antibody against VEGF, was shown to prolong survival in a phase III clinical trial in renal cell cancer and was efficient in two randomized clinical trials investigating the treatment of metastatic colorectal cancer [81].

ZD6474 is an orally bioavailable inhibitor of VEGF receptor-2 tyrosine kinase activity that in preclinical studies has been shown to inhibit both VEGF-induced signaling in endothelial cells and tumor-induced angiogenesis. ZD6474 produced significant broad-spectrum antitumor activity in a panel of human tumor xenografts [82,83]. The results obtained so far with inhibitors of angiogenesis suggest that these are novel molecules, currently in development could be useful for the treatment of chemoteraputic resistant TGCTs and to increase patients survival.

CONCLUSIONS AND PERSPECTIVES

Both environmental and genetic factors play an important role in the development of human testicular seminomas . These factor cause the deregulation of the normal differentiation processes of PGC. The incidence of seminomas has been increasing over the last decades. Remarkably, differences in incidence between adjacent countries such as Sweden and Finland are still largelyunexplained, calling for further studies. Diagnosis is usually based on identification of histological subgroups. In recent years, immunohistochemistry with a panel of suitable markers, including OCT3/4, SOX2, SOX17, HMGA1, and HMGA 2, PATZ1, GPR30 and others has given further advantages to discriminate between subgroups.

A unique characteristic of seminoma is their sensitivity to treatment. Although the better responses of seminomas versus nonseminomas is well reported, as the frequent recurrence of mature teratomas in residual treatment-resistant tumors highlighting the need for more effective therapies in these resistant forms. A deeper understanding of the molecular mechanisms underlying the development of TGCTs may provide new tools to specifically target neoplastic cells and could contribute to overcome acquired and intrinsic chemotherapy resistance. Promising molecules capable to selectively target neoplastic cells, that is, the Aurora-B serine–threonine kinases, TKs, HMGAs and proangiogenic factors inhibitors, already under clinical evaluation will open a new scenario for seminomas treatment.

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REFERENCES

[1] Chieffi, P. Molecular targets for the treatment of testicular germ cell tumors. *Mini Rev. Med. Chem.*, **2007**, *7*, 755–9.

[2] Chieffi P.; Franco, R.; Portella G. Molecular and cell biology of testicular germ cell tumors. *Int. Rev. Cell Mol. Biol.*, **2009**, *278*, 277-308.

[3] Oosterhuis, J.W.; Looijenga, L.H. Testicular germ-cell tumors in a broader perspective. *Nat. Rev. Cancer* **2005**, *5*, 210-22.

[4] Ulbright, T.M. Germ cell neoplasm of the testis. *Am. J. Surg. Pathol.*, **1993**, *17*, 1075–91.

[5] Chaganti, R.S.; Houldsworth, J. Genetics and biology of adult human male germ cell tumors. *Cancer Res.*, **2000**, *60*, 1474–82.

[6] Jones, R.H.; Vasey, P.A. Part II: testicular cancer—management of advanced disease. *Lancet Oncol.*, **2003**, *4*, 738–47.

[7] Shelley, M.D.; Burgon, K.; Mason, M.D. Treatment of testicular germ-cell cancer: a cochrane evidence-based systematic review. *Cancer Treat. Rev.*, **2002**, *28*, 237–53.

[8] Al Ghamdi, A.M.; Jewett, M.A. Stage I nonseminomatous germ cell tumours: the case for management by risk stratification. *Can. J. Urol.*, **2005**, *12*, 62–5.

[9] Classen, J.; Souchon, R.; Hehr, T.; Bamberg, M. Treatment of early stage testicular seminoma. *J. Cancer Res. Clin. Oncol.*, **2001**, *127*, 475–81.

[10] Perrotti, M.; Ankem, M.; Bancilla, A.; deCarvalho, V.; Amenta, P.; Weiss, R. Prospective metastatic risk assignment in clinical stage I nonseminomatous germ cell testis cancer: a single institution pilot study. *Urol. Oncol.*, **2004**, *22*, 174–7.

[11] Bonner, M.R.; McCann, S.E.; Moysich, K.B. Dietary factors and the risk of testicular cancer. *Nutr. Cancer*, **2002**, *44*, 35–43.

[12] Dieckmann, K.P.; Pichlmeier, U. Is risk of testicular cancer related to body size? *Eur. Urol.*, **2002**, *42*, 564–9.

[13] Dieckmann, K.P.; Hartmann, J.T.; Classen, J.; Lüdde, R.; Diederichs, M.; Pichlmeier, U. Tallness is associated with risk of testicular cancer: evidence for the nutrition hypothesis. *Br. J. Cancer*, **2008**, *99*, 1517–21

[14] Dieckmann, K.P.; Hartmann, J.T.; Classen, J.; Diederichs, M.; Pichlmeier, U. Is increased body mass index associated with the incidence of testicular germ cell cancer? *J. Cancer Res. Clin. Oncol.*, **2009**, *135*, 731–8.

[15] Garner, M.J.; Birkett, N.J.; Johnson, K.C.; Shatenstein, B.; Ghadirian, P.; Krewski, D.; Canadian Cancer Registries Epidemiology Research Group Dietary risk factors for testicular carcinoma. *Int. J. Cancer*, **2003**, *106*, 934–41.

[16] McGlynn, K.A.; Quraishi, S.M.; Graubard, B.I.; Weber, J.P.; Rubertone, M.V.; Erickson, R.L.; Persistent organochlorine pesticides and risk of testicular germ cell tumors. *J. Natl. Cancer Inst.*, **2008**, *100*, 663–71.

[17] Martin, O.V.; Shialis, T.; Lester, J.N.; Scrimshaw, M.D.; Boobis, A.R.; Voulvoulis, N. Testicular dysgenesis syndrome and the estrogen hypothesis: a quantitative metaanalysis. *Environ. Health Perspect*, **2008**, *116*, 149–57.

[18] Garner, M.; Turner, M.C.; Ghadirian, P.; Krewski, D.; Wade, M. Testicular cancer and hormonally active agents. *J. Toxicol. Environ. Health B Crit. Rev.*, **2008**, *11*, 260–75.

[19] Strohsnitter, W.C.; Noller, K.L.; Hoover, R.N.; Robboy, S.J.; Palmer, J.R.; Titus- Ernstoff, L.; Kaufman, R.H.; Adam, E.; Herbst, A.L; Hatch, E.E. Cancer risk in men exposed *in utero* to diethylstilbestrol. J. *Natl. Cancer Inst.*, **2001**, *93*, 545–51.

[20] Dieckmann, K.P.; Endsin, G.; Pichlmeier, U. How valid is the prenatal estrogen excess hypothesis of testicular germ cell cancer? A case control study on hormone-related factors. *Eur. Urol.*, **2001**, *40*, 677–83.

[21] Ekbom, A.; Richiardi, L.; Akre, O.; Montgomery, S.M.; Sparen, P. Age at immigration and duration of stay in relation to risk for testicular cancer among Finnish immigrants in Sweden. *J. Natl. Cancer Inst.*, **2003** *95*, 1238–40.

[22] Montgomery, S.M.; Granath, F.; Ehlin, A.; Sparen, P.; Ekbom, A. Germ-cell testicular cancer in offspring of Finnish immigrants to Sweden. *Cancer Epidemiol. Biomarkers Prev.*, **2005**, *14*, 280-2.

[23] Forman, D.; Oliver, R.T.; Brett, A.R.; Marsh, S.G.; Moses, J.H.; Bodmer, J.G.; Chilvers, C.E.; Pike, M.C. Familial testicular cancer: a report of the UK family register, estimation of risk and an HLA class 1 sib-pair analysis. *Br. J. Cancer*, **1992**, *65*, 255–62.

[24] Lutke Holzik, M.F.; Rapley, E.A.; Hoekstra, H.J.; Sleijfer, D.T.; Nolte, I.M.; Sijmons, R.H. Genetic predisposition to testicular germ-cell tumours. *Lancet Oncol.*, **2004**, *5*, 363–71.

[25] Skakkebaek, N.E. Possible carcinoma-in-situ of the testis. *Lancet*, **1972**, *2*, 516–7.

[26] Wicha, M.S.; Liu, S.; Dontu, G. Cancer stem cells: an old idea—a paradigm shift. *Cancer Res.*, **2006**, *66*, 1883–90.

[27] Ulbright, T.M. Protocol for the examination of specimens from patients with malignant germ cell and sex cord-stromal tumors of the testis, exclusive of paratesticular malignancies: a basis for checklists. *Arch. Pathol. Lab. Med.*, **1999**, *123*, 14–9.

[28] Berthelsen, J.G.; Skakkebaek, N.E.; von der Maase, H.; Sorensen, B.L.; Mogensen, P. Screening for carcinoma in situ of the contralateral testis in patients with germinal testicular cancer. *Br. Med. J.*, **1982**, *285*, 1683–6.

[29] Nielsen, H.; Nielsen, M.; Skakkebaek, N.E. The fine structure of possible carcinoma in-situ in the seminiferous tubules in the testis of four infertile men. *Acta Pathol. Microbiol. Scand. A.*, **1974**, *82*, 235–48.

[30] Skakkebaek, N.E.; Berthelsen, J.G.; Giwercman, A.; Müller, J. Carcinoma-in-situ of the testis: possible origin from gonocytes and precursor of all types of germ cell tumours except spermatocytoma. *Int. J. Androl.*, **1987**, *10*, 19–28.

[31] Muller, J.; Skakkebaek, N.E.; Nielsen, O.H.; Graem, N. Cryptorchidism and testis cancer. Atypical infantile germ cells followed by carcinoma in situ and invasive carcinoma in adulthood. *Cancer*, **1984**, *54*, 629–34.

[32] Almstrup, K.; Hoei-Hansen, C.E.; Wirkner, U.; Blake, J.; Schwager, C.; Ansorge, W.; Nielsen J.E.; Skakkebaek, N.E.; Rajpert-De Meyts, E.; Leffers, H. Embryonic stem cell-like features of testicular carcinoma in situ revealed by genome-wide gene expression profiling. *Cancer Res.*, **2004**, *64*, 4736–43.

[33] Hoei-Hansen, C.E.; Almstrup, K.; Nielsen, J.E.; Brask Sonne, S.; Graem, N.; Skakkebaek, N.E.; Leffers H.; Rajpert-De Meyts, E. Stem cell pluripotency factor NANOG is expressed inhuman fetal gonocytes, testicular carcinoma in situ and germ cell tumours. *Histopathol.*, **2005**, *47*, 48–56.

[34] Looijenga, L.H. Human testicular (non)seminomatous germ cell tumours: the clinical implications of recent pathobiological insights. *J. Pathol.*, **2009**, *218*, 146–62.

[35] Rajpert-De Meyts, E.; Bartkova, J.; Samson, M.; Hoei-Hansen, C.E.; Frydelund-Larsen, L.; Bartek, N.E.; Skakkebaek, N.E. The emerging phenotype of the testicular carcinoma in situ germ cell. *APMIS*, **2003**, *111*, 267–78.

[36] Chieffi, P.; Battista, S.; Barchi, M.; Di Agostino, S.; Pierantoni, G.; Fedele, M.; Chiariotti, L.; Tramontano, D.; Fusco, A. HMGA1 and HMGA2 protein expression in mouse spermatogenesis. *Oncogene*, **2002**, *21*, 3644-50.

[37] Franco, R.; Esposito, F.; Fedele, M.; Liguori, G.; Pierantoni, G.; Botti, G.; Tramontano D.; Fusco A.; Chieffi P. Detection of high mobility group proteins A1 and A2 represents a valid diagnostic marker in post-puberal testicular germ cell tumours. *J. Pathol.*, **2008**, *214*, 58–64.

[38] Fedele, M.; Franco, R.; Salvatore, G.; Baronetto, M.P.; Barbagallo, F.; Pero, R.; Chiariotti L.; Sette C.; Tramontano D.; Chieffi G.; Fusco A.; Chieffi, P. PATZ1 gene has a critical role in the spermatogenesis and testicular tumours. *J. Pathol.*, **2008**, *215*, 39–47.

[39] Esposito, F.; Boscia, F.; Franco, R.; Tornincasa, M.; Fusco, A.; Kitazawa, S.; Looijenga, L.H.; Chieffi, P. Down-regulation of estrogen receptor-β associates with transcriptional coregulator PATZ1 delocalization in human testicular seminomas. *J. Pathol.*, **2011,** *224*. 110-120.

[40] Chieffi, P.; Troncone, G.; Caleo, A.; Libertini, S.; Linardopoulos, S.; Tramontano, D.; Portella G. Aurora B expression in normal testis and seminomas. *J. Endocrinol.*, **2004**, 181, 263–70.

[41] Esposito, F.; Libertini, S.; Franco, R.; Abagnale, A.; Marra, L.; Portella, G.; Chieffi P. Aurora B expression in post-puberal testicular germ cell tumours. *J. Cell. Physiol.*, **2009**, *221*, 435–9.

[42] Barbagallo, F.; Baronetto, M.P.; Franco, R.; Chieffi, P.; Dolci, S.; Fry, A.M.; Geremia R.; Sette C. Increased expression and nuclear localization of the centrosomal kinase Nek2 in human testicular seminomas. *J. Pathol.*, **2009**, *217*, 431–41.

[43] de Jong, J.; Stoop, H.; Dohle, G.R.; Bangma, C.H.; Kliffen,M.; van Esser, J.W.; van den Bent M.; Kros, J.M.; Oosterhuis, J.W.; Looijenga, L.H. Diagniostic value of OCT3/4 for pre-invasive and invasive testicular germ cell tumours. *J. Pathol.*, **2005**, *206*, 242–9.

[44] Looijenga, L.H.; Stoop, H.; de Leeuw, H.P.; de Gouveia Brazao, C.A.; Gillis, A.J.; van Roozendaal, K.E.; van Zoelen, E.J.; Weber, R.F.; Wolffenbuttel, K.P.; van Dekken, H.; Honecker, F.; Bokemeyer, C.; Perlman, E.J.; Schneider, D.T.; Kononen, J.; Sauter, G.; Oosterhuis, J,W. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. *Cancer Res.*, **2003**, *63*, 2244–50.

[45] Atlasi, Y.; Mowla, S.J.; Ziaee, S.A.; Gokhale, P.J.; Andrews, P.W. OCT4 spliced variants are differentially expressed in human pluripotent and non-pluripotent cells. *Stem Cells*, **2008**, *26*, 3068–74.

[46] Ledford, H. Doubts raised over stem-cell marker. *Nature (London)* **2007**, 449, 647.

[47] Lengner, C.J.; Camargo, F.D.; Hochedlinger, K.; Welstead, G.G.; Zaidi, S.; Gokhale, S. Oct4 expression is not required for mouse somatic stem cell self-renewal. *Cell Stem Cell*, **2007**, *1*, 403–15.

[48] Liedtke, S.; Enczmann, J.; Waclawczyk, S.; Wernet, P.; Kogler, G. Oct4 and its pseudogenes confuse stem cell research. *Cell Stem Cell*, **2007**, *1*, 364–6.

[49] de Jong, J.; Looijenga, L.H. Stem cell marker OCT3/4 in tumor biology and germ cell tumor diagnostics: history and future. *Crit. Rev. Oncog.*, **2006**, *12*, 171–203.

[50] Avilion, A.A.; Nicolis, S.K.; Pevny, L.H.; Perez, L.; Vivian, N.; Lovell Badge, R. Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev.*, **2003**, *17*, 126–40.

[51] Boiani, M.; Scholer, H.R. Regulatory networks in embryo-derived pluripotent stem cells. *Nat. Rev. Mol. Cell Biol.*, **2005**, *6*, 872–84.

[52] Yamaguchi, S.; Kimura, H.; Tada, M.; Nakatsuji, N.; Tada, T. Nanog expression in mouse germ cell development. *Gene Expr. Patterns*, 5, 639–646.

[53] Yates, A., Chambers, I., 2005. The homeodomain protein Nanog and pluripotency in mouse embryonic stem cells. *Biochem. Soc. Trans.*, **2005**, *33* (Part 6), 1518–21.

[54] Hart, A.H.; Hartley, L.; Parker, K.; Ibrahim, M.; Looijenga, L.H.; Pauchnik, M.; Chow, C.W.; Robb, L. The pluripotency homeobox gene NANOG is expressed in human germ cell tumors. *Cancer*, **2005**, *104*, 2092–8.

[55] de Jong, J.; Stoop, H.; Gillis, A.J.; van Gurp, R.J.; van de Geijn, G.J.; Boer, M.; Hersmus, R.; Saunders, P.T.; Anderson, R.A.; Oosterhuis, J.W.; Looijenga, L.H. Differential expression of SOX17 and SOX2 in germ cells and stem cells has biological and clinical implications. *J. Pathol.*, **2008**, *215*, 21–30.

[56] Korkola, J.E.; Heck, S.; Olshen, A.B.; Reuter, V.E.; Bosl, G.J.; Houldsworth, J.; Chaganti, R.S. *In vivo* differentiation and genomic evolution in adult male germ cell tumors. *Genes Chromosomes Cancer*, **2008**, *47*, 43–55.

[57] Kim, I.; Saunders, T.L.; Morrison, S.J. Sox17 dependence distinguishes the transcriptional regulation of fetal from adult hematopoietic stem cells. *Cell*, **2007**, *130*, 470–83.

[58] Gashaw, I.; Grummer, R.; Klein-Hitpass, L.; Dushaj, O.; Bergmann, M.; Brehm, R.; Grobholz, R.; Kliesch, S.; Neuvians, T.P.; Schmid, K.W.; von Ostau, C.; Winterhager, E. Gene signatures of testicular seminoma with emphasis on expression of ets variant gene 4. *Cell. Mol. Life Sci.*, **2005**, *62*, 2359–68.

[59] Gashaw, I.; Dushaj, O.; Behr, R.; Biermann, K.; Brehm, R.; Rübben, H.; Grobholz R; Schmid, K.W.; Bergmann, M.; Winterhager, E. Novel germ cell markers characterize testicular seminoma and fetal testis. *Mol. Hum. Reprod.*, **2007**, *13*, 721–7.

[60] Staibano, S.; Franco, R.; Mezza, E.; Chieffi, P.; Sinisi, A.; Pasquali, D.; Errico, M.E.; Nappi, C.; Tremolaterra, F.; Somma, P.; Mansueto, G.; De Rosa G. Oestrogen receptor Beta, PCNA, P53, and DNA ploidy: are they markers of biological behavior in ovarian granulosa cell tumours? *Histopathol.*, **2003**, *43*, 254-62.

[61] Sinisi, A.A.; Chieffi, P.; Pasquali, D.; Kisslinger, A.; Staibano, S.; Bellastella, A.; Tramontano, D. EPN a novel epithelial cell line derived from human prostate tissue. *In vitro Cell. Dev. Biol.- An.*, **2002,** *38*, 165-72.

[62] Picascia A., Stanzione R., Chieffi P., Kisslinger A., Dikic I., Tramontano D. Pyk2 regulates proliferation and differentiation of prostate cells. *Mol. Cell. Endocrinol.*, **2002**, *186*, 81-87.

[63] Franco, R.; Boscia, F.; Gigantino, V.; Marra, L.; Esposito, F.; Ferrara, D.; Pariante, P.; Caraglia, M.; Minucci, S.; Chieffi, P. GPR30 is over-expressed in post puberal testicular germ cell tumors. *Cancer Biol. Ther.*, **2011**, *11*, 609-13.

[64] Vitale, G; Dicitore, A.; Sciortino, G.; Abbruzzese, A.; Persani, L.; Santini, D. Role of GPR30 in testicular germ cell tumors: a potential new anticancer target. *Cancer Biol. Ther.*, **2011**, *11*, 733-5.

[65] Bernstein, E.; Kim, S.Y.; Carmell, M.A.; Murchison, E.P.; Alcorn, H., Li, M.Z.; Mills, A.A.; Elledge, S.J.; Anderson, K.V.; Hannon, G.J. Dicer is essential for mouse development. *Nat. Genet.*, **2003**, *35*, 215–7.

[66] Voorhoeve, P.M.; le Sage, C.; Schrier, M.; Gillis, A.J.; Stoop, H.; Nagel, R.; Liu, Y.P.; van Duijse, J.; Drost, J.; Griekspoor, A.; Zlotorynski, E.; Yabuta, N.; De Vita, G.; Nojima, H.; Looijenga, L.H.; Agami, R. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell*, *2006*, *12*, 1169–81.

[67] Carmena, M.; Earnshaw, W.C.; The cellular geography of aurora kinases. *Nat. Rev. Mol. Cell Biol.*, **2003** *4*, 842–54.

[68] Portella G., Passaro C., Chieffi P. Aurora B: a new prognostic marker and therapeutic target in cancer. *Curr. Med. Chem.*, **2011**, *18*, 482-96.

[69] Mayer, F.; Stoop, H.; Sen, S.; Bokemeyer, C.; Oosterhuis, J.W.; Looijenga, L.H.J. Aneuploidy of human testicular germ cell tumors is associated with amplification of centrosomes. *Oncogene*, **2003**, *22*, 3859–66.

[70] Katayama, H.; Brinkley, W.R.; Sen, S. The Aurora kinases: role in cell transformation and tumorigenesis. *Cancer Metastasis Rev.*, **2003**, *22*, 451–64.

[71] Chieffi, P.; Franco, R.; Fulgione, D.; Staibano S. PCNA in the testis of the frog, *Rana esculenta*: a molecular marker of the mitotic testicular epithelium proliferation. *Gen. Comp. Endocrinol.*, **2000,** *119*, 11-6.

[72] Krause, D.S.; Van Etten, R.A. Tyrosine kinases as targets for cancer therapy. *N. Engl. J. Med.*, **2005**, *353*, 172–87.

[73] Kemmer, K.; Corless, C.L.; Fletcher, J.A.; McGreevey, L.; Haley, A.; Griffith, D.; Cummings, O.W.; Wait, C.; Town, A. Heinrich, M.C. KIT mutations are common in testicular seminomas. *Am. J. Pathol.*, **2004**, *164*, 305–13.

[74] Palumbo, C.; van Roozendaal, K.; Gillis, A.J.; van Gurp, R.H.; de Munnik, H.; Oosterhuis, J.W.; van Zoelen, E.J.; Looijenga, L.H. Expression of the PDGF alpha-receptor 1.5 kb transcript, OCT-4, and c-KIT in human normal and malignant tissues Implications for the early diagnosis of testicular germ cell tumours and for our understanding of regulatory mechanisms. *J. Pathol.*, **2002**, *196*, 467–77.

[75] Manova, K.; Nocka, K.; Besmer, P.; Bachvarova, R.F. Gonadal expression of c-kit encoded at the W locus of the mouse. *Development*, **1990**, *110*, 1057–69.

[76] Looijenga, L.H.; de Leeuw, H.; van Oorschot, M.; van Gurp, R.J.; Stoop, H.; Gillis, A.J.; de Gouveia Brazao CA, Weber RF, Kirkels WJ, van Dijk T, von Lindern M, Valk P, Lajos G, Olah E, Nesland JM, Fosså SD, Oosterhuis JW. Stem cell factor receptor (c-KIT) codon 816 mutations predict development of bilateral testicular germ-cell tumors. *Cancer Res.*, **2003**, *63*, 7674–8.

[77] Heinrich, M.C.; Blanke, C.D.; Druker, B.J.; Corless, C.L. Inhibition of KIT tyrosine kinase activity: a novel molecular approach to the treatment of KIT-positive malignancies. *J. Clin. Oncol.*, **2002**, *20*, 1692–703.

[78] Folkman, J. New perspectives in clinical oncology from angiogenesis research. *Eur. J. Cancer*, **1996**, *32*, 2534–9.

[79] Ferrara, N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr. Rev.*, **2004**, *25*, 581–611.

[80] Gasparini, G.; Longo, R.; Toi, M.; Ferrara, N. Angiogenic inhibitors: a new therapeutic strategy in oncology. *Nat. Clin. Pract. Oncol.*, **2005**, *2*, 562–77.

[81] Ranieri, G.; Patruno, R.; Ruggirei, E.; Montemurro, S.; Valerio, P.; Ribatti, D. Vascular endothelial growth factor (VEGF) as a target of bevacizumab in cancer: from the biology to the clinic. *Curr. Med. Chem.*, **2006**, *1*, 1845–7.

[82] Zakarija, A.; Soff, G. Update on angiogenesis inhibitors. *Curr. Opin. Oncol.*, **2005**, *6*, 578–83.

[83] Lee, D. Phase II data with ZD6474, a small-molecule kinase inhibitor of epidermal growth factor receptor and vascular endothelial growth factor receptor, in previously treated advanced non-small-cell lung cancer. *Clin. Lung Cancer*, **2005**, *7*, 89–91.

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