GENERAL REVIEW

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Testicular cancer stem cell hypothesis – diagnostic and therapeutic implications

Hipoteza stem ćelija testikularnog karcinoma – dijagnostičke i terapijske implikacije

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Introduction

Testicular cancer is an uncommon malignancy in men and is curable in most instances. There is marked geographical variation with age-standardized rate (ASR) *per* 100,000 ranging from 4.6 in developed countries to 0.8 in developing countries [1]. The incidence of testicular cancer is highest in Western and Northern Europe, Australia, and North America, while the lowest incidence is in Asia and Africa [2]. Despite its low overall incidence, it is the most common cancer in young men, in the third or fourth decade of life [3]. The incidence of testicular cancer has been increasing over the past four decades, while mortality rate has been decreasing in most European countries [4–6]. Over 71,000 new testicular cancer cases and 9,500 deaths are estimated to occur worldwide in 2018 [7].

According to the World Health Organisation, testicular tumours can be pathologically classified into seven categories: germ cell tumours derived from germ cell neoplasia *in situ*, germ cell tumours unrelated to germ cell neoplasia *in situ*, sex-cord stromal tumours, tumour containing both germ cell and sex-cord stromal elements, miscellaneous tumours of the testis, haematolymphoid tumours, and tumours of collecting duct and rete testis [8]. More than 90% of all testicular cancers are germ-cell tumours almost equally divided in seminomas and nonseminomas [9]. Seminomas are consisted of classic seminoma, spermatocytic seminoma and intratubular germ cell neoplasia [10]. Seminomas are composed of transformed germ cells that closely resemble the primordial germ cells (PGCs) or gonocytes that are blocked in differentiation and can not undergo normal spermatogenesis. Non-seminomas include embryonal carcinomas, yolk sac tumours, teratomas and choriocarcinomas [10]. Nonseminomas can contain different histological elements due to pluripotency of the PGCs or gonocytes, normally only apparent after fertilization (Figure 1) [11]. Seminomas are most frequent in the fourth decade of life, while nonseminomas peak in the third decade of life [11].

Testicular cancer has some unique biological features different from other solid tissue cancers. First, it has unusual histology with components that mimic any tissue type of the body. It can be explained by the fact that testicular cancer originates from germ cells that use two different types of cell division (mitosis and meiosis) [12]. Second, germ cells preserve embryonic stem cell features and pluripotency for a long period during development [12].

Aetiology and pathogenesis

Testicular cancer has a largely unexplained aetiology. There are a number of risk factors for testicular cancer associated with prenatal or perinatal exposures, including low birth weight, low maternal parity, cryptorchidism, infertility, family history, and white race. A systematic review and meta-analysis of perinatal factors in relation to

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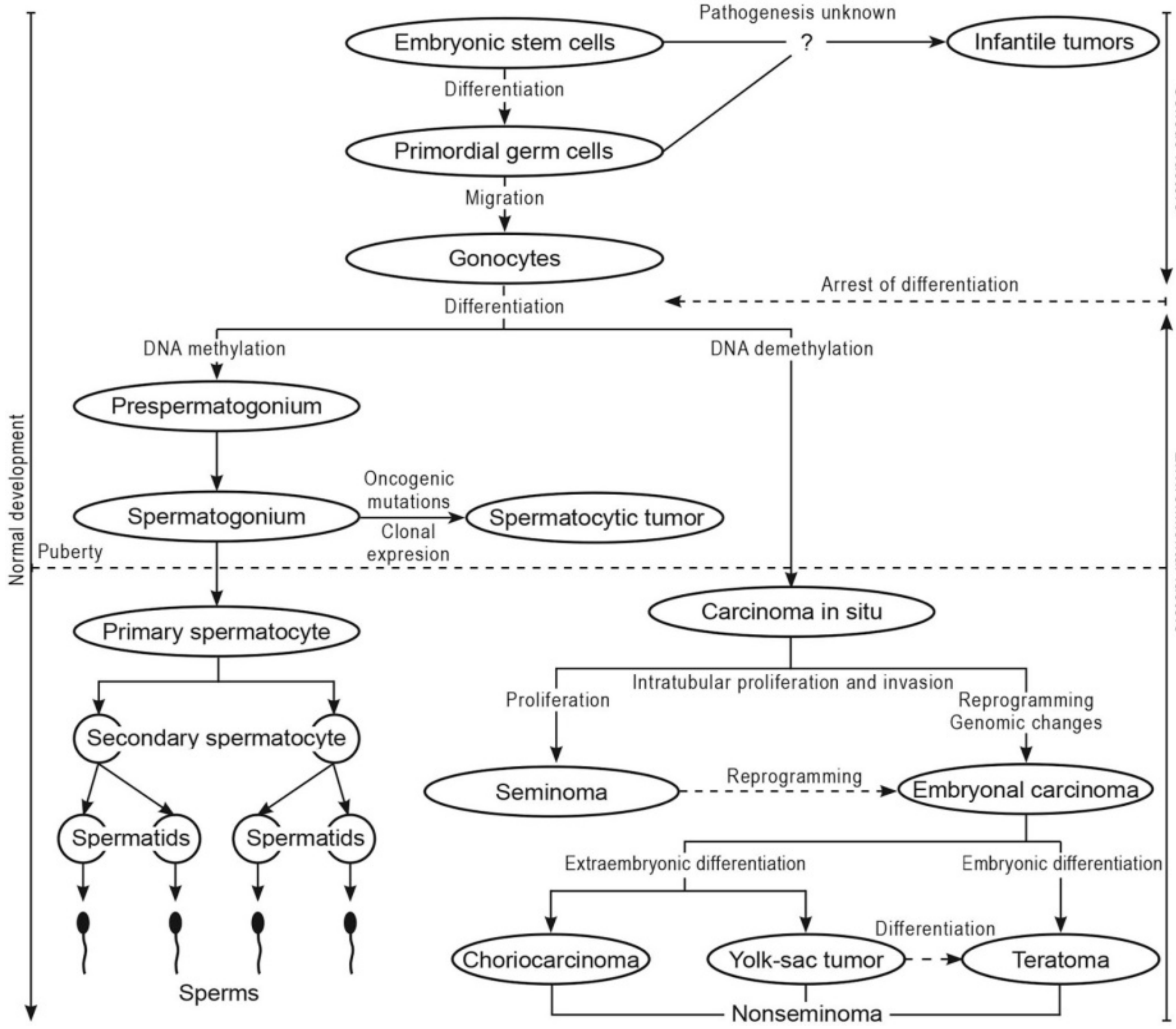

Fig. 1 – Normal germ cell development and model of histogenesis of the testicular germ-cell tumors.

the risk of testicular cancer found evidence that cryptorchidism, inguinal hernia, twinning, birth weight, and gestational age are associated with increased risk of testicular cancer [13]. It is speculated that oxidative stress can also be a cause [14, 15]. However, while spermatozoa are much more susceptible, spermatogonia are highly tolerant to oxidative stress [16, 17].

During testicular carcinogenesis preinvasive cells gave rise to overt tumour. Testicular cancer is derived from cells in the germ cell lineage that are blocked in maturation. The pre-invasive stage of testicular germ-cell tumour (TGCT) of adolescents and young adults is carcinoma *in situ* (CIS) or intratubular germ cell neoplasia unclassified, which is thought to arise from malignant transformation of a PGCs or gonocytes [18]. Skakkebaek [19] described atypical spermatogonia in testicular biopsies from two patients who later developed overt testicular tumours. There is evidence that approximately 50% of patients diagnosed with CIS of the testis develop invasive testicular cancer within 5 years of diagnosis [20]. The incidence of testicular tumours that develop from CIS has increased during last decades. There are three other very rare types of TGCT which develop without CIS stage: spermatocytic seminoma, teratomas, and yolk sac tumours that have remained at steady low incidence level [21].

Normal germ cell development

PGCs in mammals are committed to secure transmission of genetic information to the next generation by production of mature oocytes in females and spermatozoa in males. They arise at the base of allantois at an early stage of embryogenesis in week 5 to 6. Thereafter, they migrate toward both genital ridges to the places where the gonads will develop. When they rich gonadal ridges, they are called gonocytes. Embryonic germ cells are characterized by several markers including placental alkaline phosphatase (PLAP), octamer-binding transription factor (OCT4), and NANOG. Differentiation of the gonocytes into either oogonia or prespermatogonia depends on chromosomal constitution and microenvironment. PGCs are in fact the totipotent stem cell population of the body [22]. PGCs are considered the stem cells of oogenesis in female and spermatogenesis in male.

Stem cells

Stem cells are undifferentiated cells that possess ability to self-replicate and to differentiate into mature cells of the organ in which it resides [23, 24]. There are three groups of stem cells, ie., embryonic, germinal and somatic stem cells. Embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst and are the precursors of all cells in our body. Germinal stem cells in the adult produce eggs and sperm and are responsible for reproduction. Somatic stem cells are responsible for normal tissue renewal [25].

The different types of stem cells proliferate differently. ESCs divide symmetrically, whereby each daughter cell retains the properties of the parental cells resulting in a logarithmic expansion of cells [25]. Germinal and somatic stem cells divide asymmetrically, whereby one daughter cell remains a stem cell (self-renewal), and undergoes expansion and further differentiation into mature cells, whereas the other daughter cell becomes progenitor cell that undergoes expansion and further differentiation into mature cells [25].

Stem cells can be obtained from the embryo or from extraembryonic tissues such as the umbilical cord blood obtained at birth, the amniotic fluid, and the placenta. Stem cells can be found in adult mammals in bone marrow, blood, skin, and testis. ESCs are thought to be pluripotent with ability to differentiate into a variety of cell types. ESCs exposed to certain conditions differentiate into cell types of all three germ layers (endoderm, ectoderm and mesoderm) as well as into germ line cells. Adult stem cells (ASCs) are more conservative in their proliferation and differentiation. Adult stem cells are limited to the tissue in which they reside [26]. Stem cells have much longer life span and therefore have greater opportunity to gather genetic mutations [27]. It has been postulated that stem cells can be transformed into cancer if signalling pathways that regulate their renewal become disrupted.

Cancer stem cells

There are two models of carcinogenesis, “stochastic” and “stem cell”. In “stochastic” model of carcinogenesis any cell may be target of random mutation. Growth of tumours is assigned to the serial acquirement of genetic events that resulted in the turning on the genes promoting proliferation, turning of genes inhibiting proliferation, and surrounding of genes responsible for apoptosis [28]. The “stem cell” model suggests that cancers originate in tissue stem cells through deregulation of self-renewal processes. In this model of carcinogenesis, the key event is disruption of genes responsible for self-renewal [28]. The idea that precursors for a growing list of cancers are cancer stem cells (CSCs) is almost 150 years old [29]. It has been speculated that tumours are initiated and maintained by a population of cancer cells with stem cell properties known as CSCs. CSCs were first described in acute myeloid leukemia [30]. Surface markers were used to distinguish the stem cells from the rest of cells with limited proliferative potential [30]. The CSCs hypothesis became essential for understanding the carcinogenesis and developing strategies for cancer prevention. CSCs are characterized as a minor cell population able to sustain themselves by self-renewal and to generate committed progenitors that gradually form solid tumour. CSCs model is based on the idea that the vast majority of tumour cells have moderate proliferative potential compared to a small cell population – the CSCs, which are able to self-renew and proliferate in order to maintain tumour cell mass [31]. Consequently, cancer is a disease of deregulated self-renewal of normal stem cells [32]. In this model, tumour restitution and even metastases may happen due to residual chemotherapy resistant cells [32]. Normal stem cells and cancer cells share several important properties like the ability of self-renewal, activation of antiapoptotic pathways, and the ability to migrate and metastasize [29]. Stem cells are subjected to the multiple mutations required for carcinogenesis during their life cycle. Adolescent women exposed to atomic bomb radiation in Hiroshima and Nagasaki developed breast cancer 2 to 3 decades after exposure. It is thought that in that period, the mammary gland has the highest number of stem cells [33].

There is accumulating evidence that CSCs exist in a spectrum of tumours. It has been hypothesised that testicular CIS cells resemble CSCs in our body [21]. CIS cell is pluripotent and has capability to develop into variety of germ cell tumours. There is evidence that CIS is precursor of TGCT. Frequent finding of CIS in testicular parenchyma surrounding invasive cancer, as well as the development of invasive TGCT in patients in whom CIS has previously been diagnosed support hypothesis. It has been hypothesized that arrest in development and differentiation of the early germ cell lineage is the main pathogenetic mechanism that leads to neoplastic transformation into CIS [21]. These cells are localised in the seminiferous tubules located between the basal membrane and the Sertoli cell layer. CIS can be found in all risk populations for TGCT more frequently, as well as in the surrounding tissue of TGCT than in testes of healthy men. CIS cells are thought to be remnants of undifferentiated foetal cells. Soon after their discovery, similarity to gonocytes was noted. Imunohistochemical studies found that CIS cells and foetal germ cells contain large amounts of glycogen and PLAP, the most commonly used marker for detection of CIS cells.

The precise mechanism underlying the transformation of the gonocyte to CIS and further into overt testicular tumour is mainly unknown. It is thought that testicular cancer is initiated during foetal development. It was hypothesized that CIS cells originate from PGCs or gonocytes that have failed to differentiate into spermatogonia as a consequence of endocrinological imbalances [12]. PGCs are thought to be changed either during migration to the embryonic genital ridges or after cells have arrived at the gonads. Little is known about the behaviour of CIS cells after birth. It is likely that they are inactive during infancy, starting to replicate after puberty, possibly as a consequence of new hormonal conditions progressing to overt tumours. Prevalence of CIS in the general population of young adults is not known. It has been estimated that the prevalence of CIS is the same as lifetime risk of testicular cancer [34].

Diagnostic approach

"Stem cell" model for cancer will likely improve diagnosis and treatment of cancer. If the CSC hypothesis is valid, than we need to discover new tumour markers made by CSCs for early detection of cancer. Testicular cancer is potentially fatal and its treatment has severe side-effects. Therefore, efforts should be made to establish diagnosis at early, preinvasive CIS stage. At present, there is no imaging technique or serological method for the diagnosis of CIS which is asymptomatic. It can be diagnosed only by a surgical biopsy [18, 35]. CIS cells can be routinely identified in biopsies by morphologic and immunohistologic characteristics. Appropriate fixatives (Stieve's or Bouins solution) should be used to diagnose CIS cells in paraffin sections. Formalin fixation should be avoided [36]. Morphologically, CIS cells are located in a single row at the basement membrane of seminiferous tubules. Cells are larger in diameter containing larger nucleus than that of normal spermatogonia. There is a large amount of glycogen in the cytoplasm of CIS cells. Therefore, their cytoplasm appears optically empty on histological sections since glycogen is washed out during fixation.

Additional immumohistochemical staining by using several antibodies like PLAP [37] or OCT3/4 [38] is an advanced option for detecting CIS cells. The monoclonal antibodies M2A and 43/F are highly sensitive for detecting CIS immunohistochemically [39]. The immunohistochemical tumour markers TRA 1-60 [40] and neuron specific enolase [41] are expressed in the majority of CIS cells. The proto-oncogene c-kit protein product Kit is over expressed by CIS cells [42]. The possible foetal origin of CIS is supported by immunohistochemical studies of proteins present in CIS, that are also present in PGCs and gonocytes [21]. Tra-1-60 and M2A which are present in great quantity in CIS but undetected in the adult testis were also detected in normal foetal and infantile germ cells [43]. Kit is strongly expressed in early foetal germ cells up to 12 weeks of gestation [36]. Differentiation of gonocytes into infantile spermatogonia begins around 20 weeks of gestation and in some cases end prenatally, but quite often continues in the early postnatal period until 6 to 9 months of age, when the markers that are shared by PGC, gonocytes, and CIS (PLAP, OCT4, NANOG) are finally down-regulated [21, 43]. Persistent expression of these markers later in childhood is not normal [44].

Therapeutic implications

The CSCs hypothesis opens new possibilities for cancer prevention and treatment, as well as predicting the cases at high risk for metastasis [45]. Traditionally, drugs used for cancer treatment are directed against proliferating cells. Testicular cancers are among the most sensitive solid cancers to chemotherapy [46, 47]. However, it is likely that agents selectively killing CSCs are overlooked [46, 48]. Consequently, tumour growth is reinitiated and relapse is plausible. If transformed stem cells are targets of intervention, then treatments that can reduce stem cell number might reduce cancer risk. It can be achieved through induction of apoptosis or differentiation of stem cells. In acute myeloid leukemia and breast cancer, tumorigenic cells are minor part of the tumour bulk. Furthermore, new cancer model has significant impact on our ability to identify individuals at risk for metastasis. It has been hypothesized that only when CSCs disseminate self-renew metastases will occur. Further studies should develop diagnostic tools that will allow us to predict in which cases metastatic disease will develop. It will help clinicians to identify patients that will benefit from chemotherapy and spare patients from unnecessary treatment [49].

Conclusion

CIS is considered precursor of TGCT, and an excellent example of CSCs. Understanding the biology and cellular chemistry of CIS is important for developing new strategies for prevention and treatment of TGCT. Efforts should be made to obtain diagnosis of TGCT at the CIS stage, as early intervention is warranted before an invasive tumour develops. Further research is needed to obtain a method of noninvasive CIS detection. It would make possible to offer to patients timely and optimal treatment.

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Conflict of Interest

The authors have no conflict of interest to declare.

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