Int J Clin Exp Pathol 2018;11(9):4622-4629
www.ijcep.com /ISSN:1936-2625/IJCEP0079908

Original Article

Testicular germ cell tumors: a clinicopathological and immunohistochemical analysis of 145 cases

Ting Zhang[1*], Lixuan Ji[1*], Bin Liu[1], Wenbin Guan[2], Qiang Liu[3], Yuping Gao[1]

Departments of [1]Assisted Reproduction, [2]Pathology, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China; [3]Department of Pathology, Renji Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China. [*]Equal contributors and co-first authors.

Received May 11, 2018; Accepted July 31, 2018; Epub September 1, 2018; Published September 15, 2018

Abstract: Testicular germ cell tumor (TGCT) is the most common tumor usually occuring in males between 20-40 years old. In recentyears, the incidence of TGCTs has risen markedly. The outcome depends on its pathologic type and tumor stage. This study was a retrospective analysis of the clinicopathological and immunohistochemical of 145 TGCTs. According to our findings, teratoma, both mature and immature, mostly involved children, as did yolk sac tumor. Patients under 18 years old all survived for at least five years, while the mortality rate was 87.8% after five years since surgery and 96.5% in adults after three years. There was no difference between the survival rate of seminomatous tumor and non-seminomatous tumor. We also analyzed two routine diagnostic markers-PLAP and OCT4 on all TGCT tissues. Results showed that OCT4 may be a better predictive marker than PLAP to distinguish the TGCTS. Our finding suggested that TGCTs mostly occurred in young adult, and the mortality of those who under 18 years old is lower than the adult patients. However in distinguishing the seminoma tumor from non-seminoma tumor, new marker should be further study.

Keywords: Testicular germ cell tumor, PLAP, OCT4

Introduction

Testicular germ cell tumor (TGCT) is the most common tumor which usually occurs in males between 20-40 years old [1, 2]. TGCTs account for 1%~1.5% in male neoplasms and in urological tumor, TGCTs account for about 5% [3, 4]. Over these years, the incidence of TGCTs has risen markedly [5]. So, it is necessary to discuss the clinical characters of TGCTs.

TGCT has 2 main histologies; seminoma (SEM) and non-seminomatous TGCT (NSE) [6]. The embryonal carcinoma, choriocarcinoma, teratoma and yolk sac tumors are included in NSE. SEM usually arise later in life, with a mean age of 35 year old when first presents compared with 25 years of age for NSE. And there are approximately 15% of TGCTs are mixed tumors, which contain both seminoma and non-seminoma elements [7].

NSE tumors are often treated with surgery and chemotherapy, and the cure rates of NSE tumors depend on the stage of the disease. The cure rate reaches up to 99% in the early stages of NSE tumors while in advanced disease stages, the cure rate decreases from 90% in those who had good prognostic category to 50% in those who had poor prognostic features [8-11].

Postpuberal TGCTs usually cause early lymph node metastases and distant metastases. Some reports showed that about 25% patients with seminoma and up to 60% NSE patients suffer from metastases [8-11], and either surgery or chemotherapy is often ineffective. About 10-20% patient with metastatic disease will not achieve complete remission, either due to a ineffective treatment or disease relapse [5].

Since the outcome of the TGCTs depends on its pathological type and tumor stage, in order to better understand the clinical characteristics of TGCTS, we reviewed a series of 145 patients with TGCTs treated in our institute over sixteen years.

Table 1. Clinical features of 145 patients with testicular germ cell tumors (TGCTs)

	Infant (<3 y)	Adolescent (3~18 y)	Adult (>18 y)
Length (cm)	4.75 (1.3~10)	4.00 (0.7~9.0)	4.0 (0.3~16)
Benign	21	9	2
Tumor	17	6	90
Total	38	15	92
	Frequency	Percentage (%)	Average Age (mean + SD)
Mature teratoma	33	23.4	36.04±7.36
Seminoma	67	45.5	36.04±10.05
Immature teratoma	3	2.1	5.86±9.64
Yolk sac tumor	16	11	2.76±4.44
Embryonal carcinoma	12	8.3	30.00±15.33
Mixed	14	9.7	30.14±8.44
Total	145	100	
	Left	Right	Total
Location	62	83	145

Table 2. Summary of immunohistochemical antibodies

Antibody	Type	Pre-treated	Dilution	Location	Detail
PLAP	Mouse anti-human	Heated	WS	Cytoplasm	DaKo (GM719102)
OCT3/4	Goat anti-IgG	Heated	0.07638889	Nucleus	SantaCruz (c-20, sc-8629)

Materials and methods

Patients

From April 2001 to September 2017, a total of 145 patients (1 month to 60 years old) with TGCTs were treated in Xinhua hospital and Renji hospital, affiliated to Shanghai Jiaotong University (Shanghai, China). Only patients with primary tumor in the Testicular site were considered. All the clinical details, laboratory and pathologic findings were collected from the department's data base. The detail of patients was showed in **Table 1**. Objectives and implications of the results were explained subsequently, clearly and institution approved written informed consent was obtained from each patient. The study protocol was approved by the Institutional Review Board of Renji hospital and Xinhua hospital. The written informed consents from patients for using their tissue specimens were also obtained.

Methods

Surgical specimens were fixed in 10% formalin and embedded in paraffin. 4 μm sections were cut and stained with H&E. Additional 4 μm sections were deparaffinized with xylene and rehydrated in a graded series of ethanol. The deparaffinized sections were then incubated with 3% H_2O_2 to inhibit the endogenous peroxidase, followed by microwave-treated or trypsin digestion for antigen retrieval before incubation with different primary antibodies, using a two-step polymer method (EnVision TM). The sections were incubated in a humid chamber at 4°C overnight after adding primary antibodies and the **Table 2** showed the details of primary antibodies used in our study. Subsequently, second antibodies were added after PBS rinse. The sections were incubated at room temperature for 30 minutes, and then colored with DAB for 15 minutes, and finally light counterstained with hematoxylin. Positive control staining were prepared using placental tissue, testicular neoplasm and gastrointestinal stromal tumor, respectively. Negative controls were performed using blocking serum in place of primary antibody. Immunohistochemical expression was graded using a semi-quantitative scoring system based on the proportion of positive cells over total cells (percent positivity) ranging from 0 to 100% where 0% was negative expression, <50% was weak expression, ≥50% was strong expression [12].

Statistical analysis

Statistical analysis was carried out with the SPSS19.0 (SPSS Inc). Data were expressed as

Table 3. The distribution of different type of TGCTs in three age groups

	Infant (<3 y)	Adolescent (3~18 y)	Adult (>18 y)	Total
Mature teratoma	21	9	3	33
Seminoma	0	2	65	67
Immature teratoma	2	1	0	3
Yolk sac tumor	14	1	1	16
Embryonal carcinoma	1	1	10	12
Mixed	0	1	13	14

Table 4. The different type of mixed TGCTs

Mixed	Cases
Se+Im+Em	1
Se+Ma+Em	1
Em+Other	2
Yo+Em	2
Se+Yo+Em	2
Im+Yo+Em	2
Se+Im	2
Ma+Em	1
Se+Em	1

Se: seminoma; Im: Immature teratoma; Em: Embryonal carcinoma; Ma: mature teratoma; Yo: Yolk sac tumor; Other: other type of tumor.

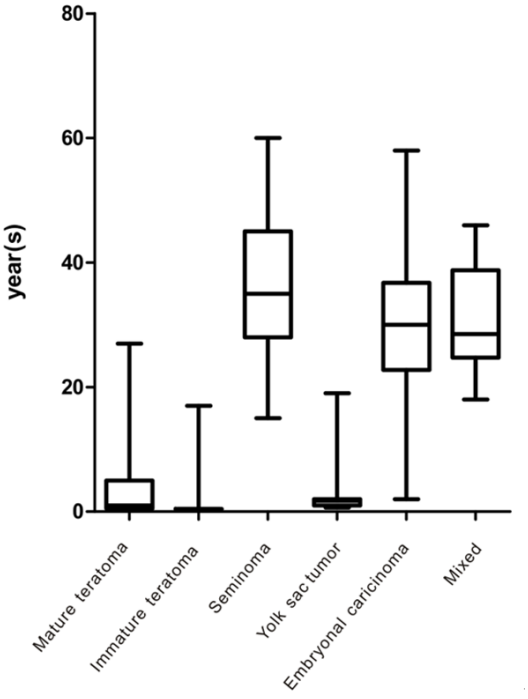

Figure 1. The average age in different type of TGCTs. 145 patients was divided as their pathological type into six groups, the age range of patients in each group was showed in the picture, the difference between each groups was analysis by Kruskal-Wallis test, P<0.001.

mean ± SEM. Specific tests, *P*-values, and sample size were reported in the figure legends or text with appropriate data.

Results

Clinical features of patients with TGCTs

From April 2001 to September 2017, a total of 145 patients with TGCTs were treated in Xinhua hospital and Renji hospital. **Table 1** shows clinical characteristics of the induced patients. Among 145 patients, Seminoma occurred predominantly, according for 45.5% (67/145), especially in adult (65/67), and mature teratoma accounted for 23.4% (34/145), immature teratoma accounted for 2.1% (3/145), Yolk sac tumor accounted for 11% (16/145), Embryonal carcinoma accounted for 8.3% (12/145) and Mix accounted for 9.7% (14/145) and there was no choriocarcinoma. 62 cases occurred at the left testicular, which accounted for 42.7% of all cases, and no bilateral lesion was detected (**Table 1**). We divided these patients into three groups according to their age. The length of tumor was comparable between three groups (median length: 4.75 cm in Infant group, 4 cm in adolescent group and 4 cm in adult group). Malignant tumor occurred predominantly in adult, according for 97.8% (90/92) of the cases. While the malignant tumor in infant and adolescent was 44.7% (17/38) and 40% (6/15) (**Table 1**).

Between three age groups, mature teratoma and yolk sac tumor occurred more frequently under age three (21/34 in mature teratoma, 14/16 in yolk sac tumor). And the seminoma and embryonal carcinoma occurred more frequently in adult (>18 years), according for 65/67 in seminoma and 10/12 in embryonal carcinoma (**Table 3**). The mixed tumor also occurred mostly in adult group (13/14), and most mixed tumor contained seminoma elements (7/14) (**Table 4**). We also analyzed the median age between different tumor types. The median age of patient with immature teratoma was 0.42 year (0.17~17.0 year), 28.5 years (18~46 years) in mixed tumor, 1.5 years (0.08~28 years) in mature teratoma, 35.5 years (15~60 years) in seminoma, 1.75 years (0.67~19 years) in yolk sac tumor and 30 years

Table 5. The five year survival data between different types of TGCTs

	Survival	Dead	Total
Imature teratoma	2	0	2
Mixed	6	0	6
Seminoma	45	7	52
Yolk sac tumor	8	0	8
Embryonal carcinoma	9	1	10
Total	70	8	78

Table 6. The three year survival data between different types of TGCTs

	Survival	Dead	Total
Imature teratoma	3	0	3
Mixed	13	0	13
Seminoma	64	2	66
Yolk sac tumor	15	0	15
Embryonal carcinoma	10	1	11
Total	105	3	108

(2~58 years) in embryonal carcinoma (**Figure 1**).

No difference of survival rate between different pathological type of TGCTs

Follow-up data of malignant tumor after surgery were collected. All the patients have done surgery and no-lymphatic metastasis was found. The follow-up data of patients who had been more than three years after surgery were available for 108 patients, one patient was out of follow up. Three patients were died after three years from surgery and eight patients were died after five years from surgery. Among the 8 patients who died after five years, seven were seminoma tumor and one was embryonal carcinoma (**Table 5**). The three patients died after three years included two seminomas and one embryonal carcinoma (**Table 6**). The five-year survival rate was 89.7% (70/78) and three-year survival data was 97.2% (105/108). No patients under eighteen years were died, so the five-year survival rate was 87.8% (58/66) and three-year survival rate was 96.5% (83/86). If we divide the patients into two groups, the five year survival rate of seminoma tumor was 86.5% (45/52) and which was 96.1% (25/26) in non-seminoma tumor group. There was no difference of survival rate between different pathological type of TGCTs ($P>0.05$, χ^2 test).

Immunohistochemistry results of PLAP and OCT4 in TGCTs

We detected the PLAP, OCT4 and other relative immune markers in all sample tissues (**Figure 2**). PLAP was located in the cell membrane and cytoplasm. It was detected in 72.3% (81/112) in malignant tumor, including 94% (63/67) in seminoma tumor, 81.3% (13/16) in yolk sac tumor, 75% (9/12) in embryonal carcinoma and 50% (7/14) in mixed tumor. No PLAP was detected in teratoma ($P<0.001$, χ^2 test) (**Table 7**).

OCT4 was detected in 70.3% (102/145) in all TGTCs, including 27% (9/33) in mature teratoma, 100% (14/14) in mixed tumor, 100% (67/67) in seminoma and 100% (12/12) in embryonal carcinoma. No OCT4 was detected in immature teratoma and yolk sac tumor ($P<0.001$, χ^2 test) (**Table 7**).

Discussion

Histologically, testicular germ cell tumors (TGCTs) are divided into Seminoma and Non-Seminoma, which are either undifferentiated, such as embryonal carcinoma, or differentiated, such as teratoma, or extra-embryonic (yolk sac choriocarcinoma) [1].

Age represents one of the most frequent factors of TGCT occurrence. The commonest age range of presentation of TGCTs, as reported, is between 20-45 years [5]. Patients rarely tend to be younger than 15 years of older than 60 years. As in our study, there was no patient above 60 years old, while 32.4% (47/145) patients were under 15 years old. Fossa et al has reported that age is an adverse prognostic factor of dead and relapse [13]. And recently, it was reported that the mortality rate was significantly increase when TGCT patients were above 40 years old after the BEP (Bleomycin + VP-16 + cisplatin) chemotherapy (HR=4.8, P=0.005) [14]. As in our study, patients under 18 years old all survive for at least five years, while the mortality rate was 87.8% after five years since surgery and 96.5% after three years in adult. However, it was reported that the five-year survival rate for patients with early-stage disease was 99% and for patients with late-stage disease was 74% [15]. The difference may due to the different geographical areas, since genetic and environmental factors play an important

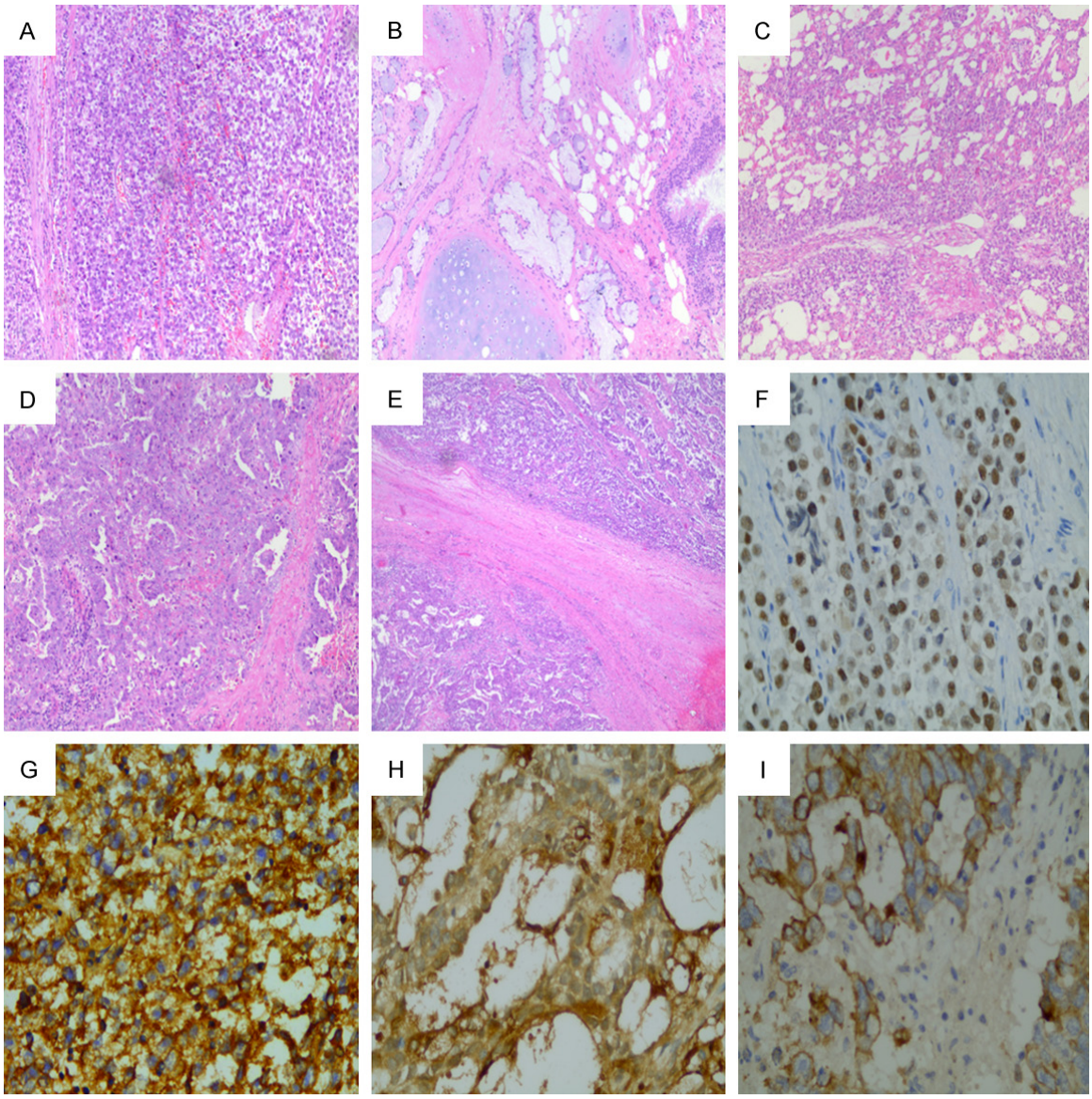

Figure 2. Pathological HE and immunohistochemical staining of TGCTs. A: Large round cells with central nuclei, prominent nucleoli and mostly clear cytoplasm defined a component of seminoma in the testicular germ cell tumor (HE×100). B: HE staining show tissues from three germ layers in Mature teratoma (HE×100). C: Yolk sac tumors are composed of clear, columnar epithelial cells arranged in sheets, cords and tubules structures (HE×100). D: Embryonal carcinomas are characterized by tumors cells organized in sheets, cord, or gland-like structures (HE×100). E: MixedTGCTs (Seminoma and Yolk sac tumor) (HE×40). F: Immunostaining for OCT in seminoma (×400). G: PLAP immunolabeling of tumor cells in seminoma (×400). H: Yolk sac tumor with AFP immonolabelling (×400). I: CK immunolabeling in mixed TGCTs (Seminoma and Yolk sac tumor) show positive staining in Yolk sac tumor and negative in seminoma (×400).

role in the development of TGCT [16]. A study of 120 cases of TGCTs reported that the five-year survival rate of seminoma tumor was higher than that of non-seminoma (90.3% vs 78.9%, $P<0.01$), which indicated that there was difference in the survival rate of different type of TGCTs [17]. While there was no difference between seminoma tumor or non-seminoma tumor in our study ($P>0.05$). This may be due to the sample size of our study was not enough.

TGCTs represent about 10% of all pediatric GCTs, but about 30% of malignant GCTs [18]. There are two age peaks of TGCTs in children: those who under three years may experience both malignant GCTs and mature teratoma,

Table 7. Summary of immunohistochemical result in different type of TGCTs

	Mature teratoma	Imature teratoma	Mixed	Seminoma	Yolk sac tumor	Embryonal carcinoma
PLAP	-	-	50%+	94%+	81.3%+	75%+
OCT4	27%+	-	+	+	-	+

The percentage of positive cases of patients was show in the table; +: 100% positive; -: 100% negative.

while adolescents may experience either seminomas or other mixed tumors [19, 20]. According to our findings, teratoma, both mature and immature mostly stroked children, so did yolk sac tumor, which is in accordance with previous reports.

Placental alkaline phosphatase (PALP) which is produced by the placenta play a big role in cellular transporting, proliferation and differentiation of cells, and in metabolism and gene transcription [21, 22]. In human, it is also produced by cancer cells [23]. PALP is not specific for testicular tumors, it can also be found in pulmonary, digestive system, breast and female reproductive organ cancers [24, 25]. However, PLAP is commonly employed as a routine diagnostic marker for seminoma/germinoma [26, 27]. It is reported that PLAP can be detected in 98% of seminomas and "carcinoma in situ" (CIS) lesions, in 85% of yolk sac tumors and 86%-97% of embryonal carcinomas [28]. In our study, it was detected 77.6% in seminoma tumor, 81.2% in yolk sac tumor, 75% in embryonal carcinoma and 50% in mixed tumor. No PLAP was detected in teratoma, which is basically in line with previous findings [27, 29].

OCT4 is an octamer binding transcription factor that has been detected in tumor germ cell which has pluripotent potential [30-34]. It is mostly detected in seminomas, embryonal carcinomas, dysgerminomas and germ cell component of gonadoblastomas. OCT4 was located in the cell nuclei [34]. Several researchers have reported that OCT4 was positive in 100% seminoma and negative in normal testicular tissues [35, 36], which was in accordance with our findings. And Hattab et al [29] also demonstrated that OCT4 is more specific and sensitive than PLAP in the diagnosis of TGCTs. Despite the 100% positive of OCT4, compared to the 92% positive of PALP, the staining background of PLAP may be so heavy that the result evaluation will be a little challenging.

In conclusion, TGCTs mostly occurred in young adult, and the mortality of those who under 18 years old is much lower than the adult patients. OCT4 may be a better prediction marker than PLAP in distinguish the TGCTS. However, in distinguishing the seminoma tumor from non-seminoma tumor, new marker should be further study.

Acknowledgements

This work was partly supported by the funding of Municipal Science and Technology Commission (17411966200).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yuping Gao, Department of Assisted Reproduction, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, 1665 Kong Jiang Road, Shanghai 200092, China. Tel: 86-18930174648; E-mail: gaoyuping@xinhuamed.com.cn

References

[1] Oosterhuis JW and Looijenga LH. Testicular germ-cell tumours in a broader perspective. Nat Rev Cancer 2005; 5: 210-222.

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

[3] La Vecchia C, Bosetti C, Lucchini F, Bertuccio P, Negri E, Boyle P and Levi F. Cancer mortality in Europe, 2000-2004, and an overview of trends since 1975. Ann Oncol 2010; 21: 1323-1360.

[4] Engholm G, Ferlay J, Christensen N, Bray F, Gjerstorff ML, Klint A, Kotlum JE, Olafsdottir E, Pukkala E and Storm HH. NORDCAN-a Nordic tool for cancer information, planning, quality control and research. Acta Oncol 2010; 49: 725-736.

[5] Vasdev N, Moon A and Thorpe AC. Classification, epidemiology and therapies for testicular germ cell tumours. Int J Dev Biol 2013; 57: 133-139.

[6] Dixon FJ and Moore RA. Testicular tumors; a clinicopathological study. Cancer 1953; 6: 427-454.
[7] Horwich A, Shipley J and Huddart R. Testicular germ-cell cancer. Lancet 2006; 367: 754-765.
[8] Chieffi P and Chieffi S. An up-date on newly discovered immunohistochemical biomarkers for the diagnosis of human testicular germ cell tumors. Histol Histopathol 2014; 29: 999-1006.
[9] Chieffi P. Potential new anticancer molecular targets for the treatment of human testicular seminomas. Mini Rev Med Chem 2011; 11: 1075-1081.
[10] Chieffi P. Molecular targets for the treatment of testicular germ cell tumors. Mini Rev Med Chem 2007; 7: 755-759.
[11] Chieffi P. Recent advances in molecular and cell biology of testicular germ-cell tumors. Int Rev Cell Mol Biol 2014; 312: 79-100.
[12] Cheng L, Sung MT, Cossu-Rocca P, Jones TD, MacLennan GT, De Jong J, Lopez-Beltran A, Montironi R and Looijenga LH. OCT4: biological functions and clinical applications as a marker of germ cell neoplasia. J Pathol 2007; 211: 1-9.
[13] Fossa SD, Cvancarova M, Chen L, Allan AL, Oldenburg J, Peterson DR and Travis LB. Adverse prognostic factors for testicular cancer-specific survival: a population-based study of 27,948 patients. J Clin Oncol 2011; 29: 963-970.
[14] Thomsen FB, Bandak M, Thomsen MF, Lauritsen J, Christensen IJ and Daugaard G. Survival and toxicity in patients with disseminated germ cell cancer aged 40 years and older. Cancer 2014; 120: 43-51.
[15] Howlader NN, Krapcho A, Miller M, Bishop D, Altekruse K, Kosary SF, Yu CL, Ruhl M, Tatalovich J, Mariotto Z, Lewis A, Chen DR, Feuer HS, Cronin EJ, KA, editors. SEER cancer statistics review, 1975-2013. NCI; Bethesda MD: 2016.
[16] Chieffi P. An up-date on epigenetic and molecular markers in testicular germ cell tumors. Intractable Rare Dis Res 2017; 6: 319-321.
[17] Svetlovska D, Miskovska V, Cholujova D, Gronesova P, Cingelova S, Chovanec M, Sycova-Mila Z, Obertova J, Palacka P, Rajec J, Kalavska K, Usakova V, Luha J, Ondrus D, Spanik S, Mardiak J and Mego M. Plasma cytokines correlated with disease characteristics, progression-free survival, and overall survival in testicular germ-cell tumor patients. Clin Genitourin Cancer 2017; 15: 411-416 e412.
[18] Chen YS, Kuo JY, Chin TW, Wei CF, Chen KK, Lin AT and Chang LS. Prepubertal testicular germ cell tumors: 25-year experience in Taipei Veterans General Hospital. J Chin Med Assoc 2008; 71: 357-361.
[19] Gobel U, Schneider DT, Calaminus G, Haas RJ, Schmidt P and Harms D. Germ-cell tumors in childhood and adolescence. GPOH MAKEI and the MAHO study groups. Ann Oncol 2000; 11: 263-271.
[20] Billmire DF. Malignant germ cell tumors in childhood. Semin Pediatr Surg 2006; 15: 30-36.
[21] Fishman WH, Anstiss CL, Pirnik MP and Driscoll SG. The exponential growth curve for the placental isoenzyme of alkaline phosphatase in sera of normal and diabetic pregnancies. Am J Clin Pathol 1973; 60: 353-358.
[22] Benham F, Cottell DC, Franks LM and Wilson PD. Alkaline phosphatase activity in human bladder tumor cell lines. J Histochem Cytochem 1977; 25: 266-274.
[23] Fishman WH, Inglis NI, Stolbach LL and Krant MJ. A serum alkaline phosphatase isoenzyme of human neoplastic cell origin. Cancer Res 1968; 28: 150-154.
[24] Manivel JC, Jessurun J, Wick MR and Dehner LP. Placental alkaline phosphatase immunoreactivity in testicular germ-cell neoplasms. Am J Surg Pathol 1987; 11: 21-29.
[25] Koshida K and Wahren B. Placental-like alkaline phosphatase in seminoma. Urol Res 1990; 18: 87-92.
[26] Hoei-Hansen CE, Sehested A, Juhler M, Lau YF, Skakkebaek NE, Laursen H and Rajpert-de Meyts E. New evidence for the origin of intracranial germ cell tumours from primordial germ cells: expression of pluripotency and cell differentiation markers. J Pathol 2006; 209: 25-33.
[27] Takeshima H and Kuratsu J. A review of soluble c-kit (s-kit) as a novel tumor marker and possible molecular target for the treatment of CNS germinoma. Surg Neurol 2003; 60: 321-324; discussion 324-325.
[28] Niehans GA, Manivel JC, Copland GT, Scheithauer BW and Wick MR. Immunohistochemistry of germ cell and trophoblastic neoplasms. Cancer 1988; 62: 1113-1123.
[29] Hattab EM, Tu PH, Wilson JD and Cheng L. OCT4 immunohistochemistry is superior to placental alkaline phosphatase (PLAP) in the diagnosis of central nervous system germinoma. Am J Surg Pathol 2005; 29: 368-371.
[30] Hansis C, Grifo JA and Krey LC. Oct-4 expression in inner cell mass and trophectoderm of human blastocysts. Mol Hum Reprod 2000; 6: 999-1004.
[31] Okamoto K, Okazawa H, Okuda A, Sakai M, Muramatsu M and Hamada H. A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. Cell 1990; 60: 461-472.

[32] Rosner MH, Vigano MA, Ozato K, Timmons PM, Poirier F, Rigby PW and Staudt LM. A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo. Nature 1990; 345: 686-692.
[33] Scholer HR, Dressler GR, Balling R, Rohdewohld H and Gruss P. Oct-4: a germline-specific transcription factor mapping to the mouse t-complex. EMBO J 1990; 9: 2185-2195.
[34] Looijenga LH, Stoop H, de Leeuw HP, de Gouveia Brazao CA, Gillis AJ, van Roozendaal KE, van Zoelen EJ, Weber RF, Wolffenbuttel KP, van Dekken H, Honecker F, Bokemeyer C, Perlman EJ, Schneider DT, Kononen J, Sauter G and Oosterhuis JW. POU5F1 (OCT3/4) identifies cells with pluripotent potential in human germ cell tumors. Cancer Res 2003; 63: 2244-2250.
[35] Cao D, Li J, Guo CC, Allan RW and Humphrey PA. SALL4 is a novel diagnostic marker for testicular germ cell tumors. Am J Surg Pathol 2009; 33: 1065-1077.
[36] Santagata S, Ligon KL and Hornick JL. Embryonic stem cell transcription factor signatures in the diagnosis of primary and metastatic germ cell tumors. Am J Surg Pathol 2007; 31: 836-845.