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Wnt suppressor and stem cell regulator TCF7L1 is a sensitive immunohistochemical marker to differentiate testicular seminoma from non-seminomatous germ cell tumor

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ABSTRACT

The accurate classification and proper identification of testicular germ cell tumors is imperative for treatment selection and clinical prognosis. Although such distinction can often be achieved by microscopic morphology alone, ancillary tests may at times be needed. T-cell factor 7 L1 (TCF7L1, also known as TCF3), a component of the Wnt signaling pathway, plays important roles in embryonic stem cell self-renewal and lineage specification. Here we examined the immunohistochemical expression and diagnostic utility of TCF7L1 in testicular germ cell tumors. Fifty cases of testicular germ cell tumors were collected, including 23 seminomas, 6 embryonal carcinomas, 1 teratoma, 1 choriocarcinoma, and 19 mixed germ cell tumors. The components of the mixed germ cell tumors were seminoma ($n = 3$), embryonal carcinoma ($n = 18$), yolk sac tumor ($n = 9$), teratoma ($n = 15$), and choriocarcinoma ($n = 4$). On immunohistochemistry of TCF7L1, only nuclear staining was considered positive. Staining was graded as negative (< 5% of tumor cells stained), minimal (5–25% positive), focal (26–50%), and diffuse (> 50%). All non-seminomatous components ($n = 54$) exhibited distinct nuclear expression of TCF7L1 (54/54; 100%). In contrast, no TCF7L1 expression was detected in the majority of seminomatous tumor component (24/26; 92%). Two seminomas (2/26; 8%) exhibited minimal weak nuclear staining (5% and 10%, respectively) for TCF7L1. In conclusion, TCF7L1, highly expressed in non-seminomatous testicular germ cell tumors, might be used as a marker for diagnosis of testicular germ cell tumors, two therapeutically different entities, for better patient management.

1. Introduction

Germ cells are derived from embryonic stem cells during embryonic development and normally programmed to become mature sperms or oocytes after puberty (Hanna and Einhorn, 2014). Oncogene activation or oncogenic mutations can cause the malignant transformation of primordial germ cells into neoplastic germ cells as a non-invasive precursor lesion, germ cell neoplasia in situ (GCNIS), or different types of invasive germ cell tumors (Cheng et al., 2017; Hanna and Einhorn, 2014; Ulbright, 2018). These GCNIS-related testicular germ cell tumors are different from non-GCNIS-related testicular germ cell tumors in their origin, molecular alteration, pathogenesis and clinical behaviors (Cheng et al., 2017; Ulbright, 2005). According to the current 2016 WHO classification, GCNIS-related germ cell tumors can be subclassified into seminoma and non-seminomatous tumors including embryonal carcinoma, trophoblastic tumor, yolk sac tumor, or postpubertal teratoma, (Ulbright et al., 2016; Williamson et al., 2017). These tumors are associated with chromosome 12p amplification and often have mixed tumor components (Shen et al., 2018). Seminomas are composed of transformed germ cells that are arrested in their development toward mature gonocytes or differentiation to a specific cell lineage. On the other hand, non-seminomatous tumors have different differentiation potentials toward a variety of embryonic, extraembryonic, and somatic tissue, but these potentials are often limited and sometimes blocked at certain stages of differentiation. Embryonal carcinoma cells have gene expression profiles similar to undifferentiated stem cells. Choriocarcinoma and yolk-sac tumor can differentiate toward extraembryonic tissues while teratoma can develop

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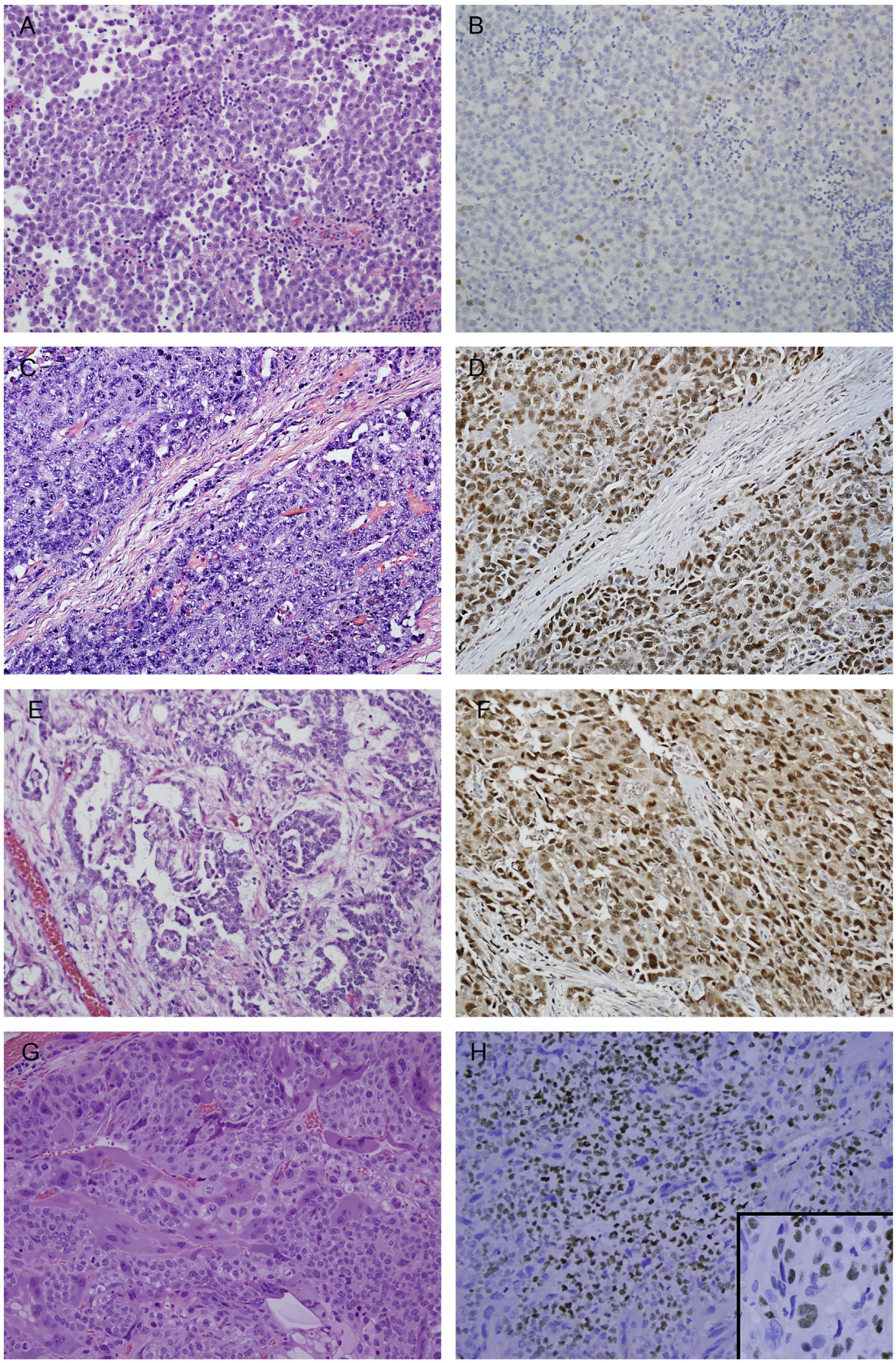

Fig. 1. Expression of TCF7L1 in seminoma, embryonal carcinoma, yolk sac tumor, and choriocarcinoma. Seminoma is negative or shows minimal positivity for TCF7L1. Seminoma is composed of sheets of dyscohesive round to polygonal cells separated by fibrous stroma with lymphocytes (A; H&E). In two positive seminoma cases, the majority of the tumor cells are negative (B; TCF7L1) with only scattered tumor cells showing weak staining positivity. Embryonal carcinoma shows strong TCF7L1 positivity. The epithelioid tumor cells arrange in sheets intermixed with trabecular and glandular growth (C; H&E) and stain intensely for TCF7L1 (D). Yolk sac tumor (E; H&E) shows strong nuclear staining for TCF7L1 (F). Choriocarcinoma has sheets of cytotrophoblasts with scattered syncytiotrophoblasts (G; H&E). Cytotrophoblasts were more widely positive for TCF7L1 than syncytiotrophoblasts (H). Original magnification: 200×.

toward endoderm, mesoderm and ectoderm elements.

GCNIS and seminoma mimic primordial germ cells and express distinct germ cell and stem cell transcriptional factors such as OCT3/4, NANOG, and SOX17 (Jones et al., 2004; Sung et al., 2006). Embryonal carcinoma also expresses OCT3/4 and NANOG but is negative for SOX17 and positive for SOX2 (Cheng et al., 2007; Santagata et al., 2007). However, these transcriptional factors are suppressed when germ cell tumors differentiate to embryonic, extraembryonic or somatic cell lineages. Therefore, these transcriptional factors may be useful diagnostic markers for classifying germ cell tumors.

The canonical Wnt signaling pathway controls pluripotent stem cell self-renewal and lineage specification (MacDonald et al., 2009). TCF7L1, a terminal component in this pathway has been shown to co-occupy promoters throughout the genome in association with OCT4 and NANOG in stem cells (Cole et al., 2008). Interestingly, TCF7L1 suppresses the expression of these pluripotency regulators and prepare stem cells for differentiation (Hoffman et al., 2013; Shy et al., 2013). Therefore, TCF7L1 may be a regulator to induce GCNIS or seminoma to differentiate toward non-seminomatous germ cell tumors. For this reason, we examined the immunohistochemical expression and diagnostic utility of TCF7L1 in testicular germ cell tumors.

2. Material and methods

2.1. Case selection

The surgical pathology files of the University of Rochester Medical Center from 2009 to 2014 were searched for cases in adult male patients with a diagnosis of testicular germ cell tumors and slides were reviewed by two pathologists (GQX and FL). This study was performed after approval by the institutional review board of the University of Rochester. Fifty cases of testicular GCNIS-related germ cell tumors were identified and classified according to the current 2016 WHO classification of germ cell tumors. These cases included 23 seminomas, 6 embryonal carcinomas, 1 postpubertal teratoma, 1 choriocarcinoma, and 19 mixed germ cell tumors. The components of the mixed germ cell tumors were seminoma ($n = 3$), embryonal carcinoma ($n = 18$), yolk sac tumor ($n = 9$), teratoma ($n = 15$), and choriocarcinoma ($n = 4$). One of the mixed germ cell tumor choriocarcinoma components was a metastasis to a lymph node.

2.2. Immunohistochemical staining

Representative archived formalin-fixed and paraffin-embedded tissue blocks of each tumor type from 50 germ cell tumors were retrieved, cut to 5-μm thick sections and mounted on charged slides. Following deparaffinization and rehydration, tissue sections were treated with 3% hydrogen peroxide (H_2O_2), to quench endogenous peroxidase activity. Antigen retrieval was performed with 10-mM citrate buffer (pH 6.0) using a pressure cooker (Pascal; Dako Cytomation, Glostrup, Denmark) for 1 min at 125 °C, followed by slow cooling. Antibody staining was conducted in a DAKO automated instrument. Five cases of germ cell tumors representing each tumor type were initially tested for rabbit monoclonal anti-TCF7L1 antibody (D15G11, #2882, Cell Signaling Technology, Danvers, MA) at 1:200, 1:400 and 1:800 dilution. All stains were performed in two batches at final antibody dilution of 1:400 with positive and negative controls. Initially tested TCF7L1 positive cases were included as negative controls by replacing TCF7L1 antibody with rabbit serum. All sections were rinsed with phosphate-buffered saline (137 mM NaCl, 2.7 mM potassium chloride, 4.2 mM sodium phosphate, and 1.5 mM potassium phosphate) and reacted TCF7L1 at 1:400 dilution for 1.5 h in phosphate-buffered saline containing 1% bovine serum albumin and 5% normal goat serum at room temperature. After washing, the sections were then incubated for 20 min with EnVision+ System horseradish peroxidase-labeled polymer conjugated with biotinylated anti-rabbit secondary antibody and 3,3′-diaminobenzidine substrate. After counterstaining with hematoxylin, slides were dehydrated and coverslipped.

2.3. Grading of immunohistochemical staining

Only nuclear staining of TCF7L1 was considered positive. The intensity of nuclear staining was graded as negative, weak, moderate, and strong. The intensity of nuclear positivity was strong in the majority of the cases, except in two seminoma cases (See results and Fig. 1). Each histologic component of pure or mixed germ cell tumors was independently evaluated and analyzed for TCF7L1 immunoreactivity in a semi-quantitative way. The percentage of positively stained tumor cells in each category was estimated. Based on the percentage of nuclear positivity, the staining was graded as: negative (< 5% of tumor cells stained), minimal (5–25%), focal (26–50%), and diffuse (> 50%).

3. Results

3.1. Expression of TCF7L1 in germ cells, sex cord stromal cells, and testicular appendages

The spermatogonia in the seminiferous tubules with either normal spermatogenesis or impaired spermatogenesis showed no detectable nuclear staining for TCF7L1, although occasional very weak cytoplasmic staining was noted. The nuclei of Leydig cells and Sertoli cells were stained negative. Epididymis and rete testis were nonreactive for TCF7L1 (data not shown).

3.2. Expression of TCF7L1 in testicular germ cell tumors

All non-seminomatous components exhibited moderate to strong positive nuclear expression of TCF7L1 (54/54; 100%). In contrast, only two seminoma cases (2/26; 8%) exhibited weak minimal staining for TCF7L. The overall sensitivity and specificity of TCF7L1 for identifying non-seminomatous germ cell tumors was 100% and 92%, respectively.

3.2.1. Seminoma

Seminoma had sheets of tumor cells separated by a reactive fibrous stroma with lymphocytes (Fig. 1A). No TCF7L1 expression was detected in the majority of seminomatous tumor components (24/26; 92%, Table 1). Two seminoma cases displayed weak nuclear staining for TCF7L1 (Fig. 1B). One was a pure seminoma case with approximately 5% positive cells, while the other one was seminomatous component from a mixed germ cell tumor with approximately 10% positive tumor cells. These positive tumor cells were randomly distributed without clustering (Fig. 1B).

3.2.2. Non-seminomatous germ cell tumors

All non-seminomatous components demonstrated variable degrees of nuclear positivity for TCF7L1 (Fig. 1D, E, and H) and most cases showed focal to diffuse nuclear staining (Table 1). Eleven of twenty-

Table 1
Expression of TCF7L1 in testicular germ cell tumors.

Tumor Subtype	Staining distribution of positively stained tumor cells			
	Negative	Minimal	Focal	Diffuse
Seminoma ($n = 26$)	24 (92%)	2 (8%)	0 (0%)	0 (0%)
Embryonal Carcinoma ($n = 24$)	0 (0%)	6 (25%)	7 (29%)	11 (46%)
Teratoma, epithelial (n = 15)	0 (0%)	12 (80%)	1 (7%)	2 (13%)
Teratoma, stromal ($n = 16$)	0 (0%)	6 (38%)	5 (31%)	5 (31%)
Yolk Sac Tumor (n = 9)	0 (0%)	3 (33%)	1 (11%)	5 (56%)
Choriocarcinoma (n = 5)	0 (0%)	2 (40%)	2 (40%)	1 (20%)

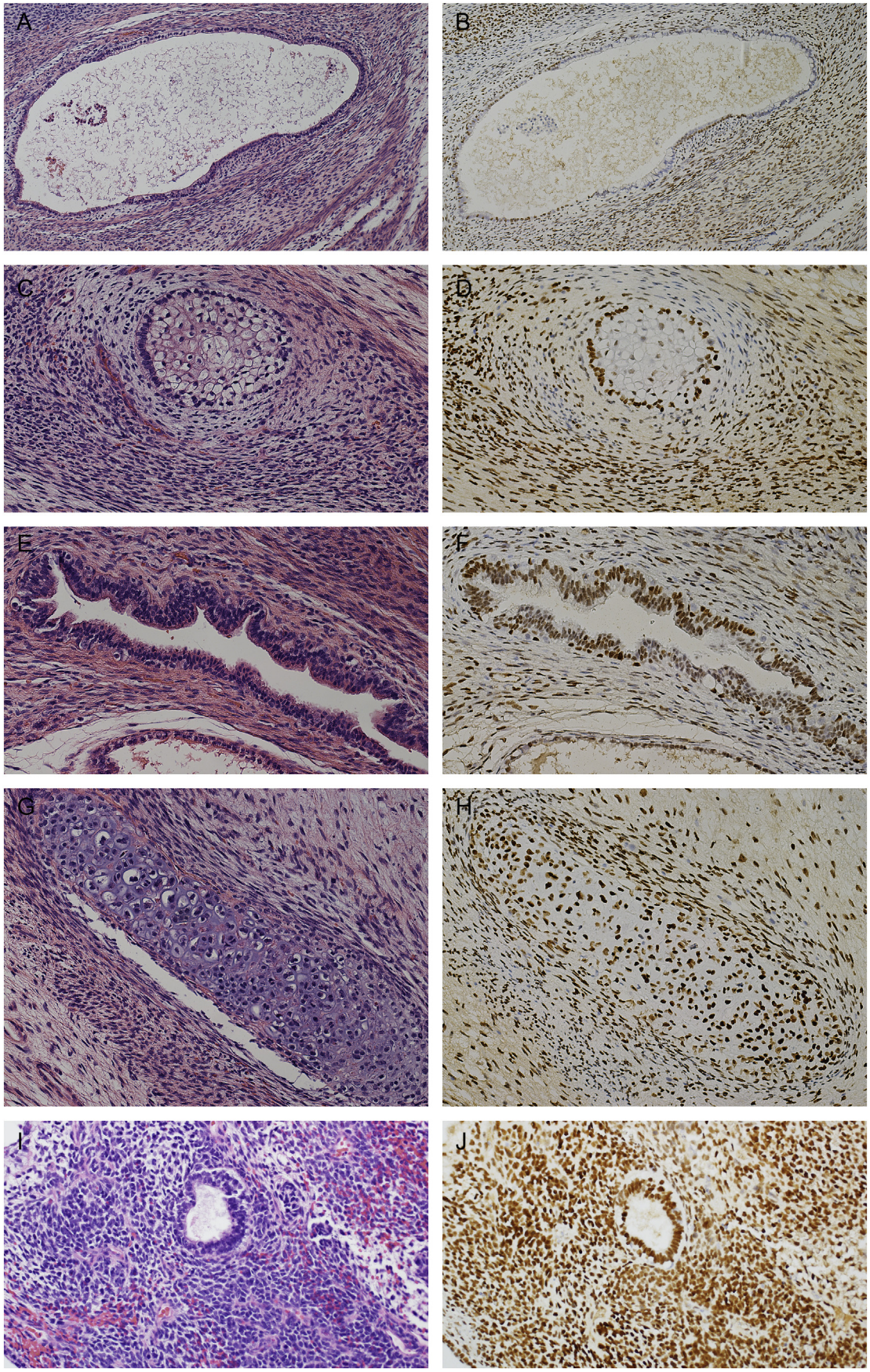

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Fig. 2. Expression of TCF7L1 in teratoma. Teratoma demonstrates TCF7L1 positivity in both mature and immature components. The mature ciliated and non-ciliated cuboidal epithelioid cells show focal moderate TCF7L1 positivity while stromal cells show focal moderate to strong staining (A and B). The squamous epithelium and stroma stain positive for TCF7L1 (C and D). The stratified and single layered cuboidal epithelium and stromal cell show reactivity to TCF7L1 (E and F). Cartilage component stains moderately positive for TCF7L1 (G and H). The immature teratoma shows strong epithelial and stromal reactivity for TCF7L1 (I and J). Original magnification 100× in A and B and 200× in B-J.

four (46%) embryonal carcinoma cases demonstrated diffuse positivity (Table 1) with moderate and strong nuclear staining (Fig. 1 C and D). Similarly, five of nine (56%) yolk sac tumor cells showed diffuse positivity (Table 1) with strong nuclear positivity for TCF7L1 (Fig. 1 E and F). The cytotrophoblasts in choriocarcinoma stained much stronger than syncytiotrophoblasts for TCF7L1 (Fig. 1 G and H). There were only rare syncytiotrophoblasts positive for TCF7L1 with weak nuclear staining (Fig. 1H boxed area). In teratoma, both mature and immature components showed reactivity for TCF7L1, while immature neuroepithelium revealed stronger nuclear reactivity than mature components (Fig. 2). Also, mesenchymal components revealed more diffuse and stronger nuclear positivity than epithelial component. Twelve of fifteen (80%) epithelial components demonstrated only minimal positivity while ten of sixteen (62%) mesenchymal components showed focal to diffuse positivity. Additionally, cartilage component showed diffuse, moderate nuclear staining for TCF7L1.

4. Discussion

GCNIS and its related germ cell tumors are derived from transformed germ cells. These neoplastic and normal primordial germ cells share some but not all transcriptional profiles with pluripotent embryonic stem cells (Cheng et al., 2017; Hanna and Einhorn, 2014; Shen et al., 2018). Germ cell tumors are broadly categorized as two clinically relevant entities: seminoma and non-seminomatous germ cell tumors (embryonal carcinoma mimicking stem cells, yolk sac tumor and choriocarcinoma with extraembryonic differentiation, and teratoma with somatic differentiation) (Cheng et al., 2017; Ulbright et al., 2016). Cytoplasmic and membranous protein products are used as earlier germ cell tumor diagnostic markers (Hanna and Einhorn, 2014; Ulbright, 2005). Placental alkaline phosphatase (PLAP) is considered as a pan-germ cell tumor marker, but it is not sensitive or specific for germ cell tumors (Uchida et al., 1981). Secreted products of germ cell tumors such as AFP and β-hCG have been broadly used as serum markers to detect yolk sac tumor and choriocarcinoma for clinical management and monitoring (Hanna and Einhorn, 2014; Ulbright, 2005). However, their routine usage in pathologic diagnosis is limited due to poor sensitivity and difficulty in interpretation (Morinaga et al., 1983). CD30 is mainly expressed in embryonal carcinoma while CD117 is positive in over 90% of seminomas (Hittmair et al., 1996; Leroy et al., 2002); neither is a germ cell tumor marker per se and both are expressed in many non-germ cell tumor tumors (Cheng et al., 2017; Ulbright, 2005).

Recent advances in stem cell biology have identified several germ cell and stem cell transcriptional factors as excellent germ cell tumor markers (Cheng et al., 2007; Ezeh et al., 2005; Santagata et al., 2007). Sal-like protein 4 (SALL4) is a sensitive pan-germ cell tumor marker, while its utility in a metastatic setting is somewhat limited as it is expressed in many poorly differentiated carcinomas (Cao et al., 2009; Miettinen et al., 2014). Additionally, SALL4 lacks specificity for individual germ cell tumor subtypes. OCT4, NANOG and SOX2 are core transcriptional factors that determine stemness for self-renewal and pluripotency. OCT4 (Jones et al., 2004; Sung et al., 2006) and NANOG (Ezeh et al., 2005; Hart et al., 2005; Hoei-Hansen et al., 2005) are expressed in seminoma and embryonal carcinoma, but not in yolk sac tumor, teratoma or choriocarcinoma. Interestingly, SOX2 is expressed in embryonal carcinoma, but not in seminoma, yolk sac tumor, teratoma or choriocarcinoma (Santagata et al., 2007). On the other hand, SOX17, a transcriptional factor controlling mesoderm development, is positive in seminoma, but negative in embryonal carcinoma, yolk sac tumor, teratoma, and choriocarcinoma (de Jong et al., 2008; Ulbright et al., 2014).

Transcriptional factors useful for identifying yolk sac tumor and choriocarcinoma are lacking. We have recently shown that ZBTB16 (PLZF) is sensitive and specific marker for yolk sac tumor (Xiao et al., 2016). In this study, we demonstrate that TCF7L1 is positive in all non-seminomatous components whether as a pure germ cell tumor or part of mixed germ cell tumors while seminoma is largely negative or only minimally and weakly positive for TCF7L1. TCF7L1 is a key member of T-cell factor/lymphoid enhancer factor (TCF/LEF) family transcription factors and acts as a transcriptional repressor in the canonical Wnt/β-catenin pathway (Cole et al., 2008; Shy et al., 2013). This pathway activation promotes pluripotent stem cell self-renewal by removing TCF7L1 from the regulatory elements of core pluripotent stem factors, such as OCT4 and NANOG, and enhancing the expression OCT4 and NANOG (Cole et al., 2008). In the absence of Wnt signaling, TCF7L1 binds to the promoters of OCT4 and NANOG and suppresses their transcription. Genetic deletion of TCF7L1 causes lineage specification defects and affects early embryonic development. These findings indicate that TCF7L1 inhibits stemness and prepares stem cells for differentiation (Cole et al., 2008). Our findings that TCF7L1 is expressed in all non-seminomatous germ cell tumors but not in seminomas, are consistent with its requirement for specification of cell lineages to generate a three-dimensional body plan during gastrulation in intact mouse embryos (Cole et al., 2008; Hoffman et al., 2013).

Seminoma can be readily diagnosed by morphology alone when it shows the classic pattern: sheets of clear cells with well-defined cytoplasmic borders and flattened, 'squared-off' nuclear membranes separated by thin lymphocyte-bearing, fibrovascular septa. However, some unusual patterns of seminomas can become diagnostic challenge. Furthermore, non-seminomatous germ cell tumors can admix or hide in a seminoma predominant mixed germ cell tumors. The significance of two seminoma cases with minimal and weak nuclear staining for TCF7L1 is not entirely clear. As TCF7L1 is transiently expressed during early embryonic development and initial differentiation of stem cells, the weak TCF7L1 expression may represent the earliest step of differentiation programs in germ and stem cells. However, both TCF7L1 positive seminoma cases were negative for cytokeratin expression and showed no morphologic evidence of differentiation. Due to the paucity of positive seminoma cases we are not able to compare TCF7L1 expression between pure seminoma and seminoma with mixed germ cell tumor to determine whether increased TCF7L1 expression in seminoma component of a mixed germ cell tumor is associated with differentiation toward non-seminomatous components.

Seminoma may sometimes contain denser, even eosinophilic or amphophilic rather than clear cytoplasm. When these are associated with nuclear atypia and crowding, seminoma can be confused as embryonal carcinoma (Ulbright, 2005). OCT4 and NANOG are not helpful to distinguish these two components as both are strongly expressed in seminoma and embryonal carcinoma. However, embryonal carcinoma is often positive for CD30, cytokeratin AE1/AE3, SOX2, but negative for SOX17, D2–40, and CD117 while the reverse pattern is true for seminoma (Hittmair et al., 1996; Lau et al., 2007; Leroy et al., 2002).

Seminoma with pseudoglandular or pseudocystic patterns may mimic glandular or cystic yolk sac tumor (Ulbright and Young, 2005). AFP is positive in approximately 60% of yolk sac tumors but is often patchy and difficult to interpret. Glypican-3 has been reported to be more sensitive than AFP for yolk sac tumor (Esheba et al., 2008). However, glypican-3 can be positive in other germ cell tumors and

somatic malignancies. ZBTB16 (PLZF), a transcription repressor, is a key regulator in normal spermatogenesis. It has been recently demonstrated that ZBTB16 (PLZF) is highly sensitive and specific for yolk sac tumor (Xiao et al., 2016).

Seminoma may contain variable amounts of syncytiotrophoblasts, which may show positivity for hCG. When syncytiotrophoblasts are forming sizable clusters, ruling out for choriocarcinoma becomes more difficult (Ulbright, 2005). The lack of the plexiform admixture of cytotrophoblasts and the absence of pleomorphism in seminoma can assist differential diagnosis. Our results show that TCF7L1 is a sensitive marker for both cytotrophoblasts and syncytiotrophoblasts while negative in seminoma.

5. Conclusions

In summary, TCF7L1 is a highly sensitive and specific marker for separating non-seminomatous germ cell tumors from seminoma. Applying TCF7L1 as a diagnostic marker can aid in the distinction of these two therapeutically different entities, yielding more accurate diagnoses and optimizing patient management.

Declaration of Competing Interest

None.

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