Utility of OCT3/4, TSPY and \(\beta\)-catenin as biological markers for gonadoblastoma formation and malignant germ cell tumor development in dysgenetic gonads

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Abstract.

BACKGROUND: Gonadoblastoma (GB) is regarded as an \textit{in situ} form of germ cell tumor in dysgenetic gonads, and 30\% of patients with GB develop a dysgerminoma/seminoma tumor.

OBJECTIVE: Determine whether OCT3/4 and \(\beta\)-catenin are expressed in dysgenetic gonads before GB development and whether TSPY participates in the OCT3/4-\(\beta\)-catenin pathways in the malignant invasive behavior.

METHODS: dysgenetic gonads of Disorders of sex differentiation (DSD) patients with mixed gonadal dysgenesis were analyzed by immunohistochemistry and immunofluorescence for comparison with GB and dysgerminoma/seminoma.

RESULTS: Our results suggest that the development of GB is secondary to the interaction of OCT3/4 and TSPY, that \(\beta\)-catenin does not participate in this process.

CONCLUSIONS: The use of this biological markers detects the potential high risk gonads.

Keywords: Gonadoblastoma, OCT3/4, TSPY, \(\beta\)-catenin, dysgenetic gonads, mixed gonadal dysgenesis

1. Background

Gonadoblastoma (GB) is regarded as an \textit{in situ} form of germ cell tumor in dysgenetic gonads (type II GCTs). This type of tumor is thought to be a precursor to seminoma/dysgerminoma tumors. It almost exclusively affects a subset of patients with disorders of sex differentiation (DSD) [5,7]. In 35\% of GB cases, overgrowth of the germinal component leads to dysgerminoma/seminoma [8]. The TSPY gene (testis-specific protein, Y encoded) localized within the GBY locus (gonadoblastoma locus on the Y chromosome) has been shown to be involved in the multistep transformation of germ cells to GB [3,13]. However, the
precise role that TSPY plays in GB development and its involvement in the malignant transformation are not clear [15]. OCT3/4 has been implicated in the GB oncogenic process, but the molecular details of OCT3/4 deregulation are still unknown [6,12]. Analysis of OCT3/4, E-cadherin and β-catenin showed that the proliferation of immature germ cells in GB may be due to the interaction between OCT3/4 and accumulated β-catenin in the nuclei of the immature germ cells, leading to the development of invasive behavior and the progression of GB into dysgerminoma/seminoma transformation were included. The use of the tissues was approved by the Institutional Bioethics Board. The analyses were performed using the classification of the World Health Organization. Formalin-fixed, paraffin-embedded sections were analyzed using immunohistochemistry and immunofluorescence (Table 1). The assays were performed in triplicate. Positive controls for β-catenin, TSPY and OCT3/4 were included in each experiment. The analyses were performed by an experienced pathologist, using the classification of the World Health Organization. The histological results were assessed by two scientists experienced in germ cell pathology (YRP and IP). Antigen-antibody complexes were detected using the avidin-biotin peroxidase method (KO679 LSAB + Sys/HRP kit, DakoCytomation, Carpinteria, CA) or with a secondary antibody conjugated to fluorescein isothiocyanate. The histological characteristics of the tissues revealed three of the four morphological patterns described by Martine Cools et al. [8]. Eight of the 18 samples were from dysgenetic testis (DT); germ cells in all 8 of the DT samples were confirmed by positive TSPY-staining. The second pattern, found in 5/18 samples, was streak tissue within undifferentiated gonadal tissue (UGT). UGT is characterized by germ cells that are not enclosed in seminiferous tubules or follicles organized in cord-like structures or by those without apparent organization. One of the UGTs contained a burnt-out gonadoblastoma. The third pattern observed in 2/18 cases was streak tissue. We also included one bilateral GB and one dysgerminoma as controls (Figs 1(A), (D), (G), (J)). In the rest of the samples, no GB or developing tumor was observed (Table 1).

### 2. Materials and methods

Eighteen paraffin-embedded tissue samples from 15 pediatric patients with mixed gonadal dysgenesis or ambiguous genitalia and a 45, X/46, XY karyotype were studied. Tissue samples from two bilateral GB and one dysgenetic gonad with dysgerminoma/seminoma transformation were included. The use of the tissues was approved by the Institutional Bioethics Board. The analyses were performed using the classification of the World Health Organization. Formalin-fixed, paraffin-embedded sections were analyzed using immunohistochemistry and immunofluorescence (Table 1). The assays were performed in triplicate. Positive controls for β-catenin, TSPY and OCT3/4 were included in each experiment. The analyses were performed by an experienced pathologist, using the classification of the World Health Organization. The histological results were assessed by two scientists experienced in germ cell pathology (YRP and IP). Antigen-antibody complexes were detected using the avidin-biotin peroxidase method (KO679 LSAB + Sys/HRP kit, DakoCytomation, Carpinteria, CA) or with a secondary antibody conjugated to fluorescein isothiocyanate. The histological characteristics of the tissues revealed three of the four morphological patterns described by Martine Cools et al. [8]. Eight of the 18 samples were from dysgenetic testis (DT); germ cells in all 8 of the DT samples were confirmed by positive TSPY-staining. The second pattern, found in 5/18 samples, was streak tissue within undifferentiated gonadal tissue (UGT). UGT is characterized by germ cells that are not enclosed in seminiferous tubules or follicles organized in cord-like structures or by those without apparent organization. One of the UGTs contained a burnt-out gonadoblastoma. The third pattern observed in 2/18 cases was streak tissue. We also included one bilateral GB and one dysgerminoma as controls (Figs 1(A), (D), (G), (J)). In the rest of the samples, no GB or developing tumor was observed (Table 1).

### Table 1

<table>
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<th>Case</th>
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<th>TSPY</th>
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<th>Co-localization</th>
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*UGT = tissue with burnt-out gonadoblastoma, DT = dysgenetic testis, SG = streak gonad, UGT = undifferentiated gonadal tissue, R = right, L = left, GB = gonadoblastoma, DG = dysgerminoma.
3. Results

In 14/18 of the gonadal samples, the germ cells stained positive for OCT3/4; OCT3/4 immunoreactivity was detected in the nuclei of immature germ cells and was observed exclusively in the DT, UGT, and control tumors as well as in GB and dysgerminoma tumors (Figs 1(C), (F), (I), (L)). OCT3/4 protein was not detected in mature germ cells or the streak tissue. TSPY immunostaining was positive in 14/18 gonads. TSPY protein staining was strongly positive in the nuclei of the germ cells in DT, UGT, GB and dysgerminoma tissues. Some protein was also detected as a faint stain in the germ cell cytoplasm (Figs 1(B), (E), (H), (K)). As in the case of GB, TSPY was detected in the UGT tissue containing burnt-out gonadoblastoma, suggesting that OCT3/4 and TSPY are key proteins in the development of GB. The samples that were negative for TSPY were mainly those with streak regions lacking germ cells. To determine whether TSPY and OCT3/4 were colocalized in the nuclei of immature germ cells, confocal microscopy was performed. It showed that both proteins were colocalized in the immature germ cell nuclei in one dysgenetic testis, in
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Fig. 2. Double-staining immunofluorescence and confocal analysis of dysgenetic testis positive for TSPY, OCT3/4 and β-catenin. (A) TSPY (green) localized in immature germ cells inside the seminiferous tubules, (B) OCT3/4 in the same tissues showing the same immunofluorescence pattern (red), (C) merged image and transmitted light micrograph optical transmission of the analyzed area indicating colocalization of both proteins in the nuclei of immature germ cells, (D) TSPY-positive immunofluorescence signal (green), (E) β-catenin (green) and (F) optical transmission and merged image showing colocalization of both proteins.

UGT and in GB (Figs 2(A)–(C)). Previous results have suggested that OCT3/4 and β-catenin participate in important steps during GB malignant transformation. β-catenin immunoreactive regions were observed only in 3/18 dysgenetic gonads. Nuclear staining in immature germ cells was observed in one DT, in UGT/GB and in GB (Figs 3(A)–(J)). Interestingly, in our previous report, dysgerminoma showed diminished β-catenin expression. Gonads that were positive for β-catenin also expressed OCT3/4 and TSPY, which colocalized. All OCT3/4, TSPY positive gonads demonstrated expression of Ki67, a cell proliferative marker (Fig. 1P-S). Confocal microscopy showed colocalization of β-catenin and OCT3/4 in all the positively stained samples, as we reported previously. TSPY colocalized with β-catenin in β-catenin-positive cells (Fig. 2D-F). However, the majority of the dysgenetic gonads tested were negative for this marker; eliminating the possibility that β-catenin participates in gonadoblastoma formation.

4. Discussion

Pure GB is regarded as an in situ form of germ cell tumor that affects almost exclusively a subset of DSD patients with dysgenetic gonads. GB does not behave as a malignant lesion; nevertheless, approximately 30% of all patients with gonadoblastoma develop a dysgerminoma/seminoma [1,7,8]. The age at diagnosis is variable, with approximately 94% of the cases being diagnosed during the second or third decades of life; we have demonstrated the presence of GB in infants [14]. It is important to identify a biological marker capable of detecting those dysgenetic gonads with a high potential for developing a tumor. Key proteins associated with germ cell tumor development, such as OCT3/4, β-catenin, TSPY and Ki67, were analyzed in 16 dysgenetic gonads and two germ cell tumors. GB originates from the surviving OCT3/4-positive germ cells within undifferentiated gonadal tissue in the dysgenetic gonad [8].

We classified our dysgenetic tissue into three patterns (DT, UGT and streak gonad). It is important to emphasize that the streak tissue must be carefully examined to identify UGT in all the patients. In our samples, five streak tissues contained UGT (Figs 1(A)–(C)). Gonadal biopsy identified as UGT or DT contained OCT3/4-positive cells, indicating a high risk for germ cell tumor formation because OCT3/4-positive cells are implicated in the GB oncogenic process. OCT3/4 is considered the most informative marker for the diagnosis of germ cell tumors [9,11]. In contrast, TSPY gene is the putative gene that predisposes dysgenetic gonads of intersex patients to develop gonadoblastomas. TSPY-positive immature germ cells


were observed in only two dysgenetic gonads, one of which was in a UGT containing a burnt-out GB. Not all OCT3/4-positive cells showed the presence of TSPY-positive nuclei, suggesting that the interaction between OCT3/4 and TSPY is an important step in GB formation. The colocalization of both proteins in the nuclei of immature germ cells, together with the Ki67 proliferative marker, supports the idea that the interaction of these two proteins in the nuclei of immature germ cell leads to cellular proliferation and GB development (Fig. 2). This finding confirms that the study of these proteins are a significant diagnostic marker for GB, CIS/ITGCNU and seminomatous tumors [1, 9]. The abundant expression of TSPY in both gonadoblastomas and CIS/ITGCNU tissues further supports the concept of a common origin [13] (Figs 1(E)–(H)). In the same way, the OCT3/4 transcription factor plays a pivotal role as a key regulator of pluripotency in the early stages of mammalian development [12]. Our observations suggest that in the dysgenetic gonad, OCT3/4 and TSPY nuclear overexpression are the key factors in the development of GB. The ectopic germ cells and the dysgenetic tissues require the presence of both proteins to proliferate. OCT3/4 expression in germ cell tumors and cancers of somatic origins suggests that it might have a proliferative function at the cellular level when it is ectopically expressed in these cells [13]. GB is not a common tumor; therefore, an insufficient number of cases have been analyzed. Previous research on β-catenin and OCT3/4 suggests that both proteins participate in the same oncogenic pathway during germ cell tumor development. The interaction between OCT3/4 and the β-catenin that accumulated in the nuclei of immature germ cells leads to the development of invasive behavior and the progression of GB into dysgerminoma/seminoma in dysgenetic gonads [14]. Here, the data show that β-catenin is expressed only in the nuclei of immature germ cells in the dysgenetic tissues that coexpressed OCT3/4 and TSPY. The remainder of the samples did not express β-catenin. This finding suggests that β-catenin participates only after the GB is established and is not involved in dysgenetic gonad progression to GB. In our previous study, we found that β-catenin expression is diminished in dysgerminoma tumors by comparison with colon adenocarcinoma; however, its colocalization with OCT3/4 suggests that both proteins participate in the same oncogenic pathway [4]. These observations distinguish β-catenin as a malignancy marker in the germ cells in which it is expressed and in dysgenetic tissues that are OCT3/4-TSPY-positive. In conclusion, dysgenetic tissue expressing OCT3/4-TSPY is associated with an extremely high risk for GB development, and both proteins are key players during GB development. The analysis of OCT4, SRY, TSPY and β-catenin expression in dysgenetic gonads may introduce modifications in the microenvironment that could contribute to a malignant transformation process.
The presence of $\beta$-catenin suggests that this protein is linked to malignant transformation (Figs 2(D)–(F)) [2]. The presence of OCT3/4-TSPY in the gonadal biopsy tissues from DSD patients is an indicator of a high risk for GB, and $\beta$-catenin should be used as a marker for malignant germ cell tumors.

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References

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