Expression analysis of the pluripotency marker UTF-1 for determining the applicability of testis-sparing surgery for prepubertal testis tumors

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ABSTRACT

Testis-sparing surgery (TSS) is widely performed for prepubertal testicular tumors because most of these tumors are benign and have biological characteristics that make them amenable to local treatment. Here, we report a case of prepubertal teratoma enucleation. In order to evaluate the proliferative potential of the tumor and the applicability of testis-sparing surgery, we investigated the expression of UTF-1—a specific marker of pluripotency and self-renewal—using real-time reverse transcription-polymerase chain reaction and immunohistochemistry. Because the enucleated tissue did not demonstrate the expression of UTF-1, the applicability of TSS was verified, in the present case.

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Prepubertal testicular tumors have different histopathology and clinical behavior compared with adult testicular cancers [1]. These tumors are also relatively uncommon, with an incidence of 0.5–2 per 100,000 boys. In contrast to adult testis tumors, the vast majority of prepubertal testis tumors contain only one histological type [2]. Although their incidence varies according to the published report [3–5], teratomas are the most prevalent prepubertal testis tumor, representing 48% of these tumors in a recent multicenter report [2]. Because several reports confirm the observation that most prepubertal testis tumors are benign [4,6], the concept of testis-sparing surgery (TSS) is widespread and accepted for these tumors.

Here, we report a case of prepubertal teratoma treated with TSS, wherein we also investigated the expression of undifferentiated embryonic cell transcription factor 1 (UTF-1)—a specific marker of spermatogonial stem cells [7,8]. UTF-1 expression was investigated within both tumor lesions and normal tissue in order to assess the malignancy potential of the tumor and the applicability of TSS.

1. Case report

A 38-month-old boy was referred to our hospital because of differences in the size of his testes. He did not show any external genitalia abnormalities at birth, and did not have any significant past history. Upon physical examination, his right scrotum was swollen and slightly indurated. There was no tenderness and the surface of right testis was smooth. The right testis measured $23 \times 17 \times 17$ mm, and the left testis was $17 \times 12 \times 12$ mm. The results of a blood analysis failed to show any abnormalities, except for slightly elevated lactate dehydrogenase levels (LDH; 260 U/mL); serum $z$-fetoprotein (AFP; 0.9 ng/mL) and human chorionic gonadotropin-beta (hCG-$\beta$; <0.1 ng/mL) levels were normal. An endocrine evaluation showed age-appropriate levels of hormones. Scrotal ultrasonography revealed a $14 \times 13 \times 10$ mm mass of mixed echogenicity, with calcification, and a hypoechoic layer surrounding a tumor in the right testis (Fig. 1A). Magnetic resonance imaging (MRI) revealed a heterogeneous mass with fluid accumulation (Fig. 1B). From these imaging findings, a right testicular tumor, such as teratoma or epidermoid cyst, was suspected. Since the testicular parenchyma adjacent to the tumor area was normal, a TSS was planned.

Under general anesthesia, an inguinal incision allowed the scrotal contents to be exposed, and spermatic cord control was achieved with a vessel loop. Following avascularization, the tunica albuginea was incised and the tumor was detected (Fig. 2A). The border between the tumor and the normal testicular parenchyma was clear, allowing the tumor to be bluntly enucleated (Fig. 2B). Because an intraoperative frozen pathological examination revealed a mature teratoma, the tunica albuginea was approximated with 5–0 absorbable sutures (Fig. 2C), and the normal testicular tissue was returned to the scrotum. A final pathological diagnosis of the mature teratoma indicated the relative amounts of tissue derived from the different germ layers, and the degree of tumor maturation (Fig. 2D); other evidence of malignancy did not exist. There were no postoperative complications, and the patient showed no local
recurrences over a 20-month follow-up period. The residual testicular parenchyma was also preserved and did not show any atrophy.

Although the etiology of testicular cancer is unknown, recent research has provided evidence that the spermatogenic stem cells and their progenitors are involved in tumorigenesis [9]. Testicular cancer comprises a heterogeneous group of neoplasms classified as either seminomas or nonseminomas, both arising from carcinoma in situ (CIS). Furthermore, CIS development is attributed to abnormalities of germline stem cells, such as gonocytes or spermatogonia [10]. Recently, we evaluated spermatogonial stem cell activity using expression of UTF-1—a stem cell marker [11]. Thus, in order to assess the malignancy potential and subsequent applicability of tumor enucleation, UTF-1 expression was investigated using real-time reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry in normal and tumor tissues of this patient. For comparison, seminoma and embryonal carcinoma tumor tissue samples from pediatric patients were also examined.

RNA was extracted from the frozen testicular tissue, preserved at $-80\,^\circ\text{C}$ immediately after removal, according to the manufacturer’s instructions [12]. Real-time RT-PCR was performed using UTF-1- and GAPDH (glyceraldehyde-3-phosphate dehydrogenase)-specific primers, as previously described [11]. The relative expression values of UTF-1 are shown in Fig. 3A. In the teratoma, UTF-1 gene expression was significantly lower, compared with normal testis and seminoma tissue ($p < 0.001$). In the immunohistochemical evaluation, UTF-1 was detectable in the germ cells of normal tissue, but not in tumorous tissue. However, the UTF-1 protein was evident in the area of a recurrent teratoma from another case (Fig. 3B).

2. Discussion

In patients with prepubertal testicular tumors, TSS is widely performed because the majority of these tumors are unifocal and benign [1–4]. TSS is also acceptable as it preserves residual

Fig. 1. (A) Scrotal ultrasonography and (B) magnetic resonance imaging (T2-weighted) showing a heterogeneous mass with a hypoechoic layer and fluid accumulation (arrowheads).

Fig. 2. (A, B, and C) Operative findings of testis-sparing surgery. Tumor (arrow) was bluntly enucleated. (D) Histologic examination of the enucleated tumor in a permanent preparation. The final pathological diagnosis was mature teratoma.
Fig. 3. (A) Relative expression values of the UTF-1 gene. In the teratomatous area, UTF-1 gene expression was significantly lower compared to those of the normal testis and seminoma (p < 0.001). (B) Hematoxylin and eosin (HE) staining and immunohistochemistry for UTF-1 (reduced from x200).
testicular function and prevents the need for subsequent hormone replacement therapy. The suitability of a tumor for TSS is determined during a preoperative examination, including specific echoic findings, tumor size, and the absence of elevated serum AFP levels [1–6]. Furthermore, the accuracy of intraoperative frozen pathological examinations in these cases is high [13].

Taskinen et al. reported 2 patients who had recurrence due to incomplete primary resection [14], demonstrating the proliferative potential of these tumors. Previously, a study of prepubertal testis tumors and their relationship to intratubular germ cell neoplasia (ITGCN) revealed that germ cells adjacent to infertile germ cell tumors are commonly proliferative [15]. ITGCN, synonymous for CIS, has been commonly accepted as a precursor for both seminoma and non-seminoma tumors in adolescents and young adults [9]. In order to identify ITGCN in the absence of morphological features, immunohistochemical markers have been established [9]. Among them is UTF-1, a novel transcription coactivator that is mainly expressed in pluripotent embryonic stem cells [11]. Although UTF-1 is used to assess spermatogonial stem cell activity [11], it is also a specific histological marker for some testicular cancers. Wang et al. reported that UTF-1 was differentially expressed in ITGCN, seminoma, and embryonal carcinoma by immunohistochemically evaluating specimens from 500 tumors [8]. In addition, UTF-1 revealed a continuous expression pattern throughout human gonadal development from fetus to adult, unlike other pluripotency markers, such as OCT-3/4 and NANOG. This characteristic may not be related to pluripotency, but rather to a high rate of proliferation and self-renewal [7]. Thus, in the present case, spermatogonia could be identified in normal testis, but UTF-1 expression could not be detected in the teratoma by either real-time RT-PCR or immunohistochemistry; these results were consistent with previous reports [7,8]. On the other hand, in the recurrent teratoma lesion, UTF-1 expression was positive, suggesting the presence of undifferentiated cells. In the present case, because the enucleated tissue did not demonstrate UTF-1 expression, the applicability of TSS was verified. However, as UTF-1 is specific for undifferentiated cells with proliferative potential, UTF-1 expression is unlikely to be predictive of malignant transformation and metastasis.

3. Conclusion

A patient with prepubertal teratoma underwent TSS. To assess the malignant potential of the tumor and the applicability of TSS, the expression of UTF-1 was investigated within normal and tumor tissue from the affected testis. Because the enucleated tissue did not demonstrate UTF-1 expression, the applicability of TSS was verified in the present case. UTF-1 expression is unlikely to be a predictive factor for malignant transformation and metastasis.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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Conflict of interest
None.

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References