OCT4: A Sensitive and Specific Biomarker for Intratubular Germ Cell Neoplasia of the Testis

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ABSTRACT

Purpose: OCT4 (POU5F1, OCT3) immunostaining highlights pluripotent cells (embryonal carcinoma and seminoma) in primary testicular germ cell tumors, but its relative usefulness in diagnosing intratubular germ cell neoplasia, unclassified (IGCNU) is not well established. The present study aimed to establish OCT4 as a sensitive and specific marker for IGCNU, a putative precursor for adult germ cell tumors.

Experimental Design: We evaluated OCT4 immunostaining in 44 cases of IGCNU from patients who had testicular germ cell tumors. In addition, 27 of the 44 IGCNU sections were also examined with antibodies to placenta-like alkaline phosphatase, the most frequently used immunohistochemical marker for intratubular germ cell neoplasia. Sections from the testes of 10 patients who had undergone orchiectomy for hormonal treatment of prostate cancer and from autopsies of 10 patients without histories of germ cell tumors were also examined for OCT4 immunostaining. The immunoreactivity of the autopsy tissues was determined with vimentin staining, and all were reactive.

Results: In all 44 of the cases, antibody to OCT4 marked the nuclei of nearly all of the dysplastic cells of intratubular germ cell neoplasia but not non-neoplastic testicular cells. The staining intensity was strong in every case, and there was little or no background staining. All 20 of the control specimens (10 orchiectomy specimens from prostate cancer patients and 10 testes from autopsies) were completely negative for OCT4. The 27 cases that were stained with antiplacenta-like alkaline phosphatase antibodies showed staining of variable intensity in the areas of intratubular germ cell neoplasia, and there was a high level of background staining artifact.

Conclusions: OCT4 is a sensitive and specific maker for intratubular germ cell neoplasia.

INTRODUCTION

Intratubular germ cell neoplasia, unclassified (IGCNU) is a precursor lesion for malignant germ cell tumors of the adult testis (1–3). It is frequently found adjacent to testicular germ cell tumors (4) and is an important lesion to recognize in biopsy specimens, because it virtually always progresses to an invasive germ cell tumor if left untreated (5, 6). IGCNU is seen with increased frequency in patients at increased risk for testicular germ cell tumors, including those with cryptorchidism (7, 8), gonadal dysgenesis (9), or prior testicular germ cell tumors (10).

Although IGCNU is often clearly discernible in hematoxylin and eosin-stained sections, a reliable and sensitive immunohistochemical marker for this lesion would be useful, because IGCNU can be difficult to recognize, especially in testicular biopsies for infertility.

OCT4 (POU5F1 and OCT3) is a POU-domain, octamer-binding transcription factor that has been detected in neoplastic germ cells with pluripotent potential (11–15), including seminomas, dysgerminomas, embryonal carcinomas, and the germ cell component of gonadoblastomas. Antibody to OCT4 marks the nuclei of these pluripotent cells (15). OCT4 immunostaining has been shown previously to highlight the cells of IGCNU (15); however, this study used only a few IGCNU cases and did not discuss the findings in detail. Placental-like alkaline phosphatase (PLAP) has been shown to be a sensitive marker for IGCNU (16–21) and is the immunohistochemical marker currently used most often to help identify IGCNU in testicular biopsy and orchiectomy specimens. It, however, is not a totally specific marker for IGCNU, because isolated, non-neoplastic spermatocytes rarely show PLAP positivity (16). Immunohistochemical staining for OCT4 and PLAP in a series of specimens containing IGCNU was undertaken to assess the relative usefulness of these two markers in the diagnosis of IGCNU.

MATERIALS AND METHODS

Archival materials from 44 cases of IGCNU in patients with testicular germ cell tumors accessioned from 1991 to 2003 were retrieved from the surgical pathology files of Indiana University Hospital. All of the lesions were classified according to accepted criteria (22): IGCNU was identified morphologically on hematoxylin and eosin-stained histologic sections by virtue of seminoma-like cells with clear cytoplasm and enlarged nucleoli along the basal aspect of seminiferous tubules lacking normal spermatogenesis.

Four-micron-thick sections were cut from the paraffin blocks and stained with hematoxylin and eosin. Additional sections were obtained for immunohistochemical studies, which were performed on an automated immunostainer. We evaluated OCT4 immunostaining in all 44 of the cases of IGCNU. In addition, 27 of the 44 IGCNU specimens were also examined with antibodies to PLAP. Sections from the testes of 10 patients who had undergone orchiectomy for hormonal treatment of
prostate cancer and from 10 testes from autopsy patients without histories of germ cell tumors were also examined for OCT4 immunostaining. The immunoreactivity of the autopsy tissues was tested with immunohistochemistry for vimentin, and all of the specimens were reactive.

OCT4 immunostaining was accomplished with a polyclonal goat anti-OCT4 antibody (C20, sc 8629; Santa Cruz Biotechnology, Santa Cruz, CA; 1:500 dilution, 30 minutes at room temperature) directed toward the COOH terminus of the protein, as described previously (23–25). Antigen retrieval was carried out by heating sections in 1 mmol/L ethylene diaminetetraacetic acid (pH 8.0) using a water bath at ~95°F for 30 minutes. Endogenous peroxidase activity was inactivated by incubation in 3% H2O2 for 15 minutes. Nonspecific binding sites were blocked using Protein Block (DAKO, Carpinteria, CA) for 20 minutes.

PLAP immunostaining was accomplished with a polyclonal rabbit anti-PLAP antibody (Signet Pathology Systems, Inc., Dedham, MA; prediluted, 45-minute incubation). Antigen retrieval was carried out with citrate buffer. Vimentin immunostaining was accomplished with a monoclonal mouse antihuman vimentin antibody (Clone V9; DAKO Corporation, Carpinteria, CA; prediluted, 10-minute incubation). No antigen retrieval was required.

RESULTS
In all 44 of the specimens, OCT4 immunostaining marked the nuclei of nearly all of the atypical cells of IGCNU (Figs. 1 and 2). The staining intensity was strong in each specimen, and there was little or no background staining. Normal testicular components in the specimens did not stain with the OCT4 antibody. All 20 of the control specimens (10 orchiectomy specimens from prostate cancer patients and 10 from autopsy patients) were completely negative for OCT4. The 27 specimens that were stained with anti-PLAP antibodies all showed positive staining of variable intensity in the areas of IGCNU, and there was a high background staining (Fig. 1C). The staining intensity was greater with OCT4 immunostaining than with PLAP immunostaining in each case.

DISCUSSION
IGCNU refers to a precursor lesion that can eventuate in all types of invasive germ cell tumors of the adult testis (1–3), with the exception of spermatocytic seminoma (26). The diagnosis of this lesion is important due to its propensity to progress to a malignant germ cell tumors; Giwercman estimates that virtually all cases of IGCNU will eventually progress to an invasive germ cell tumor without intervention (6). Therefore, an immunostain specific to this lesion, such as OCT4, could be an extremely useful adjunct to hematoxylin and eosin-stained slides in difficult or less conspicuous cases of IGCNU. Certain populations of individuals are at increased risk for the development of this precursor lesion, such as patients with cryptorchid testes (even after orchiopexy; refs. 7, 8), men with sperm counts of <20 million per mL and/or testicular volume <12 mL (27, 28), patients with a history of germ cell tumors of the contralateral testicle (10), and patients with certain types of gonadal dysgenesis (e.g., 46XX/46XY; ref. 9) or the androgen insensitivity (testicular feminization) syndrome (29). Because at-risk patients may be screened for IGCNU with bilateral testicular biopsies, a sensitive and specific marker is a useful tool in examining these specimens. For patients with a history of germ cell tumors who
have IGCNU in the contralateral testis, a specific immunostain could also be useful in monitoring the response of IGCNU to chemotherapy in a post-treatment biopsy. This is important, because IGCNU is sometimes unresponsive to chemotherapy (30, 31).

Our study shows the great value of OCT4 immunohistochemistry for the identification of IGCNU. All 44 cases of IGCNU stained intensely with antibodies directed against OCT4 with no background staining and highlighting of nearly all of the neoplastic cells. Such staining was specific, with no staining of non-neoplastic germ cells.

A number of novel immunomarkers have been proposed to help in the diagnosis of IGCNU (32). We chose to compare OCT4 immunostaining with PLAP, because we feel the latter is the most widely used marker in actual clinical practice and the one that has been most widely studied as a biomarker for IGCNU (16–21). Previous studies have shown PLAP to be expressed in 93% to 98% of IGCNU cases by immunohistochemistry (16, 18, 20). Other markers, such as c-kit and TRA-1-60, have also been used in the diagnosis of IGCNU; however, these have shown less consistency than PLAP in identifying this lesion (32). For this reason and the frequent finding of only focal c-kit positivity in IGCNU (33), we did not directly compare OCT4 and c-kit immunostaining in this study.

We directly compared OCT4 and PLAP immunohistochemistry in 27 cases of IGCNU and found that OCT4 immunostaining showed greater staining intensity than PLAP immunostaining, with less background staining artifact. Because the sensitivity and specificity of PLAP immunostaining for IGCNU has already been established in the literature (16, 18, 20) and because the OCT4 staining pattern was so uniform in all 44 of the cases studied, we feel that an accurate comparison of these immunomarkers can be made with 27 cases. Both OCT4 and PLAP immunostaining showed similar sensitivity for this lesion; however, in addition to being expressed in IGCNU, PLAP positivity has also been identified in rare, non-neoplastic germ cells (16) and in many types of invasive germ cell tumors, as well as in associated syncytiotrophoblasts (16), making this marker less specific than OCT4. On the other hand, anti-OCT4 antibodies show positive staining only in seminomas, embryonal carcinomas, and IGCNU in the adult testis (15), and we found no staining of non-neoplastic germ cells, either in the index cases or the non-neoplastic controls. The strong nuclear reactivity produced by OCT4 immunostaining readily permits identification of IGCNU and would be of particular benefit in identifying this lesion in biopsy specimens from patients at risk for invasive germ cell tumors where involvement may be focal and subtle. For these reasons, OCT4 will likely prove the superior marker for IGCNU as the accumulated experience with it widens.

Looijenga et al. (15) studied OCT4 immunostaining in 100 different tumor categories and in 3,600 individual cancers using tissue microarray analysis. This study included 16 cases of IGCNU, which were identified morphologically and confirmed by double-staining with c-kit. They found all 16 of the cases to be positive for OCT4 (15). However, we do not feel that the usefulness of OCT4 immunohistochemistry in the diagnosis of IGCNU was adequately addressed, because this finding was mentioned only briefly and not the major focus of the study; it was examined in only a few cases without an adequate negative control. Thus, we confirm and extend their results by focusing only on the use of OCT4 in the diagnosis of IGCNU and by comparing our findings with the most commonly used immunomarker for this lesion and by using non-neoplastic testicular tissue as a negative control.

In conclusion, OCT4 immunostaining is a sensitive and specific marker for intratubular germ cell neoplasia, unclassified type, because nearly all of the dysplastic cells of this lesion stained positively with anti-OCT4 antibodies in all of the 44 cases examined in this study, and no staining of non-neoplastic testicular cells was detected. Staining with anti-OCT4 antibodies demonstrates consistently strong reactivity for IGCNU and little to no background staining artifact. By comparison, PLAP immunostaining shows equal sensitivity, but is less specific and shows more variable staining intensity. OCT4 immunostaining is, therefore, a useful diagnostic tool in the identification of IGCNU in the adult testis. OCT4 immunohistochemistry almost certainly will prove to be a useful adjunct to hematoxylin and

![Fig. 2 IGCNU. A, high magnification of seminiferous tubule with IGCNU. The germ cells are aligned along the basal portion of the tubule and have clear cytoplasm and enlarged, hyperchromatic nuclei with prominent nucleoli. B, the same seminiferous tubule stained for OCT4. The abnormal germ cells demonstrate a nuclear staining pattern.](https://clincancerres.aacrjournals.org/)

**Fig. 2** IGCNU. A, high magnification of seminiferous tubule with IGCNU. The germ cells are aligned along the basal portion of the tubule and have clear cytoplasm and enlarged, hyperchromatic nuclei with prominent nucleoli. B, the same seminiferous tubule stained for OCT4. The abnormal germ cells demonstrate a nuclear staining pattern.
eosin staining in identifying IGCNU in difficult cases and in at-risk patients.

REFERENCES

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