C-Kit and Bcl-2 Expression in Testicular Cancer

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Corresponding Author:
Dr. Syeling Lai,
Pathology, Pathology, Michael E. DeBakey VA Medical Center, 2002 Holcombe Blvd, 77030 - United States of America

Submitting Author:
Dr. Syeling Lai,
Pathology, Pathology, Michael E. DeBakey VA Medical Center, 2002 Holcombe Blvd, 77030 - United States of America

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Author(s): Lai S

Abstract

Alterations of C-Kit and Bcl-2 have been shown to play important roles in many types of human cancers. Although there are emerging studies of C-Kit in testicular cancer biology, the significance of Bcl-2 in testicular neoplasms has not been well understood. We studied C-Kit and Bcl-2 expressions by immunohistochemistry in 18 testicular cancers including 8 seminomas, 7 mixed germ cell tumors, 1 metastatic adenocarcinoma, 1 leiomyosarcoma and 1 large cell lymphoma. We found that C-Kit was diffusely and strongly expressed in seminomas and metastatic adenocarcinoma, while only focal weak to moderate C-Kit positivity was shown in seminomatous and nonseminomatous components of mixed germ cell tumor. Bcl-2 expression was seen in metastatic adenocarcinoma and lymphoma with focal weak reactivity in leiomyosarcoma, but not in seminoma and mixed germ cell tumors. Our findings may be helpful in differential diagnosis primary and metastatic testicular cancer and in targeted therapy.

Introduction

Testicular neoplasms constitute most common tumor type in men between 20-40 years of age (1,2). Majority of tumors are germ cell tumor (GCT). Recent developments in understanding the molecular biology of testicular cancer implicated a number of critical key players including p53, pRb, pINK protein and C-Kit. Kit is a protooncogene located on chromosome 4q11q12. Expression and gain of function mutation of the C-Kit gene that encodes a receptor tyrosine kinase (CD117) have been reported in gastrointestinal stromal tumor, seminoma, adenoid cystic carcinoma, endometrial adenocarcinoma, and esophageal squamous cell carcinoma. Kit plays a crucial role in the survival, proliferation and migration of normal primordial germ cells and gonocytes (1-3). C-Kit mutation has been found to occur early in germ cell development and gonocytes during embryogenesis with activated C-Kit receptor are restricted in their differentiation (3). Genes in apoptosis pathway B-cell CLL/lymphoma 2 (Bcl-2) was first reported to be associated with t(14:18) translocations in follicular B cell lymphoma (4). Overexpression of Bcl-2 has been reported in other malignancies including carcinoma, sarcoma, gastrointestinal stroma tumor. Few studies have been reported in testicular cancers. In this study, we assessed immunostaining panel of C-Kit and Bcl-2 expression in testicular cancers including GCT and non-GCT. We correlated expression of both markers with patient’s survival.

Methods

A retrospective chart review was conducted in patients treated with orchietomy at Michael E. DeBakey Veterans Affairs Medical Center. Prior to conducting this review, approval by the Baylor College of Medicine institutional review boards was obtained. Eighteen orchietomy specimens of testicular cancers constitute the materials. All patients presented with unilateral testicular masses. The specimens include 8 seminomas, 7 mixed germ cell tumors, 1 metastatic adenocarcinoma, 1 leiomyosarcoma and 1 large cell lymphoma. The mean patient age was 48.7 years (range 24-64 years).

Hematoxylin and Eosin (H&E) stained slides were retrieved and examined to confirm the original histopathological diagnosis and to ensure tumor adequacy. A 5 µm section was cut from Paraffin-embedded blocks and mounted on positively charged slides, deparaffinized in Bond Dewax Solution and rehydrated in descending grades (100-70%) of ethanol. Endogenous peroxide activity was blocked by pretreatment with 3% hydrogen peroxide for 10 minutes, and antigen recovery was achieved by 20 minutes heat-induced epitope retrieval. Immunohistochemical stains were performed using an automated tissue-staining system. Tissue sections were stained with C-Kit and Bcl-2 monoclonal antibodies following manufacturer’s instruction. Bound antibodies were detected using polymer reagent conjugated with horseradish peroxidase and affinity purified goat-anti-mouse antibody followed by the 3,3’-Diaminobenzidine (DAB) Chromogen Kit. Appropriate positive and negative controls were performed. The stains with weak, moderate or strong nuclear or cytoplasmic/membrane intensities in majority of stained tumor cells were scored as 1+, 2+ and 3+, respectively. Tumors with no staining in both the nucleus and cytoplasm were considered as negative staining.
Results

The testicular cancers consist of 15 GCT (8 seminoma, 7 mixed GCT) and 3 non-GCT (1 metastatic prostatic adenocarcinoma, 1 leiomyosarcoma and 1 large B-cell lymphoma). By immunohistochemistry, C-Kit is diffusely and strongly positive in all seminomas (Table 1, Figure 1). Three mixed GCT with seminomatous components displayed focal weak (2), to moderate (1) C-Kit expression, mainly in seminoma component. The other two mixed GCT without seminomatous component only revealed focal weak expression of C-Kit. No Bcl-2 staining was seen in any of the GCT. C-Kit expression was observed in one metastatic adenocarcinoma, but not leiomyosarcoma and lymphoma (Figures 2 & 3). In contrast to GCT, all three non-GCT showed Bcl-2 staining (strong and diffuse in 1 metastatic adenocarcinoma, 1 lymphoma, and focal weak expression in leiomyosarcoma. C-Kit/Bcl-2 +/- pattern was seen in 100% of GCT with available blocks, compared with C-Kit/Bcl-2 +/- pattern in 67% non-GCT. In 3 to 10 years available patient’s follow-up, two patients died (1 patient with mixed GCT died 3 months, and 1 with metastatic adenocarcinoma died 3 years after diagnosis).

Discussion

Germ cell tumors including seminoma and nonseminomas (NSGCT) are most common testicular tumors. C-kit mutation is thought to be related to tumor carcinogenesis. Its expression was found in puberty and could be detected in adult spermatogenesis at low level. Steiner et al. showed Kit expression in Leydig cells and spermatogonia of contralateral testes in all patients with NSGCT indicating disturbed germ cell development associated with Kit (2). Kit is expressed in almost all intratubular germ cell neoplasia, most seminomas and some NSGCT (1,5-7). Our study showed strong expression of C-Kit in all seminomas and weak expression in mixed GCT, which is in keeping with the findings by other studies (1,2,8). Negative staining of Bcl-2 in all germ cell tumors in our study supports the previous publications that apoptosis inhibiting gene Bcl-2 was predominately down regulated in GCT (9,10). Bcl-2 is detected in all three cases of non-GCT.

The histological features on H&E sections are sufficient for the diagnosis of majority of testicular tumors. Immunohistochemical studies may aid in diagnosis in some situations. Reported C-kit positivity frequency varies from 90-100% (11,12). C-Kit expression is not highly specific in GCT. Interestingly, we found strong C-Kit reactivity in one case of prostatic adenocarcinoma metastatic to testis. This indicates that metastatic prostatic adenocarcinoma may aberrantly express C-Kit. Metastasis needs to be considered in differential diagnosis if morphology is not typical for GCT in C-kit positive tumor. Additional markers such as OCT3/4, AE1/AE3 and EMA in conjunction with C-Kit and Bcl-2 may be helpful to confirm the diagnosis.

Previous report has showed no correlation of loss of C-Kit expression with prognosis (11). Only two patients died in the current study during the period of available follow-up and both showed strong C-kit expression and negative Bcl-2 activity. Our study contains limitation in a retrospective analysis. The sample size is small in a single institution. A larger study performed in a multi-institutional setting would provide important statistic value of gene expression in prognosis. Further studies of Kit mediated carcinogenesis is necessary to search targeted therapy for C-kit dependent cancer.

In summary, C-Kit and Bcl-2 showed differential expression profile in testicular GCT and non-GCT. A combination of both markers helps differential diagnosis of GCT and non-GCT. The limited data in our series cannot indicate statistic significance of the association between the expression of any of these genes and survival.

References

Illustrations

Illustration 1

Figure 1. A-C, Seminoma. 1A, Hematoxylin and eosin section. 1B, Diffuse positive C-Kit immunostaining. 1C, lack of immunoreactivity with Bcl-2.

Illustration 2

Figure 2. A-C, Metastatic adenocarcinoma. 2A, Hematoxylin and eosin section. 2B and 2C, Positive immunostaining with C-Kit and Bcl-2.
Illustration 3

Figure 3. A-C, Large B-cell lymphoma. 3A, Hematoxylin and eosin section. 3B, Negative immunostaining with C-Kit. 3C, Diffuse positivity with Bcl-2.

Illustration 4

Table 1

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Table 1. C-Kit and Bcl-2 expression in testicular germ cell and non-germ cell tumors.

GCT, germ cell tumor; NS, not available; med. ACA, medullary adenocarcinoma; L, lymphocytic component; M, mixed seminomas component.