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SHORT COMMUNICATION

Expression of cellular apoptosis susceptibility (CAS) in the human testis and testicular germ cell tumors

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Abstract

Testicular germ cell tumors are the most frequent malignancies found in men between 15 and 44 years old. Although cellular apoptosis susceptibility (CAS) was demonstrated to be upregulated in breast cancer and colon cancer, the expression of CAS in the human testis and testicular germ cell tumors remained elusive. In the present study, CAS-positive signals were detected in the normal testicular tissues, cancer adjacent normal testicular tissues, seminoma, yolk sac tumor, and teratoma. Interestingly, the expression level of CAS in testicular germ cell tumors (TGCTs) (but not seminoma) was significantly lower than that of human testicular tissues and cancer adjacent normal testicular tissues, suggesting that decreased CAS contributed to the progression of TGCTs. Notably, the expression of CAS in seminoma was significantly higher than that of in the non-seminomas, consistent with the results from TCGA database. Furthermore, the localization of CAS is mainly restricted in the nucleus in the lesions of normal human testicular tissue and cancer adjacent normal testicular tissue. Although the expression of CAS was not significantly different between normal testicular tissue and seminoma, CAS was more enriched in cytoplasm in seminoma compared to the normal, cancer adjacent tissue and other types of TGCTs. The current results demonstrated reduced expression of CAS in the human testicular germ cell tumors and the CAS translocation from the nuclear to cytoplasm in seminoma, thereby supporting a possible role in normal testis function and in the development of seminoma.

Keywords CAS · Testicular germ cell tumors

Introduction

Testicular germ cell tumors (TGCTs) are the most frequent malignant cancer among men between 14 and 44 years of age, and its incidence has risen over the past two decades around the world [1]. Typically, TGCTs are classified into seminomas and non-seminoma based on their histological composition. Seminoma is originated from undifferentiated germ cells and is homogenous with a poorly invasive ability. Non-seminomas, including embryonal carcinoma, teratoma, yolk sac tumor, and choriocarcinoma, are derived from undifferentiated (embryonal carcinoma) and differentiated multipotent cells (teratoma, yolk sac tumor, and choriocarcinoma), which have more aggressive phenotype with a heterogeneous histology [2].

Cellular apoptosis susceptibility (CAS) is encoded by the human homolog of the essential yeast chromosome segregation gene 1 (CSE1), and regulates cancer cell apoptosis and has thereby been implicated in many tumor progressions [3]. Tomoaki et al. demonstrated that nuclear CAS regulated the

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transcriptional activity of p53 which can determine different cellular outcomes such as growth arrest or cell death [4]. A recent study showed that CAS was overexpressed in thyroid carcinoma and maintained PTC cell survival [5]. Another study also reported that high expression of CAS in HCC associated with macroangioinvasion and knockdown of CAS could inhibit the cell migration and invasion by deregulation of integrins [6]. Knockdown of CAS in colorectal cancer could reduce tumorigenesis [7, 8]. However, the expression of CAS in reproductive diseases is conflicting. CAS was found to be highly expressed in breast cancer [9] and ovarian cancer [10] and promotes cancer metastasis [11, 12]. In the contrary, our previous findings showed that the expression of CAS was downregulated in the endometriotic lesions [13], indicating the different roles of CAS in reproductive-related diseases. Interestingly, CAS is a novel interacting protein of CD147 identified from germ cells [13]. However, the expression pattern of CAS in the normal human testis and TGCTs and its implication to the progression of TGCTs remain elusive.

In the present study, we investigated the expression pattern of CAS. We initially observed decreased expression of CAS in embryonal carcinoma, yolk sac tumor, and teratoma. Although there was no significant difference of expressed CAS between normal, cancer adjacent samples and seminomas tissue, the cytoplasmic CAS was dramatically increased in seminomas compared to the normal and other types of TGCTs, indicating that the high expression of CAS in cytoplasm might be involved in the development of seminoma.

Materials and methods

Specimens

A tissue microarray was purchased from US Biomax, Inc (Rockwell MD), which contained 102 samples totally. Specifically, the specimens contain 5 samples of testicular normal tissues, 13 samples of cancer adjacent normal testicular tissues, 45 samples of seminoma, 27 samples of non-seminomas including 15 samples of embryonal carcinoma, 8 samples of yolk sac tumor, and 4 samples of teratoma.

Immunohistochemistry

Immunohistochemistry was performed according to the instructions of the immunohistochemical kit (SP-9001, ZSGB-BIO, Beijing, China). In brief, slides were rehydrated and underwent antigen-retrieval with EDTA antigen repair solution (1:49) for 20 min. After PBS rosined (three times/5 min), quenching of endogenous peroxidase activity by incubation of the sections in 3% H_2O_2 for 10 min, blocking in normal goat serum block reagent (SP 9001, ZSGB-BIO, Beijing, China) for 1 h at room temperature. Then, the sections were incubated with primary antibody (anti-CAS, 1:500) at 4 °C overnight. After three washes with PBST, biotinylated secondary antibody was added and incubated for 1 h, then the sections were developed in freshly prepared diaminobenzidine (DAB) and counterstained with hematoxylin, clear and mounted for assessment under microscope.

Immunohistochemical assessment

IHC analysis is a semiquantitative analysis that contains both intensity and distribution of staining [14]. The interpretation of IHC is based on overall staining intensity (0, +, ++, +++) and the percentage of the positive tumor cells (0–25%, 26–75% and > 75%) [15]. The tissues were diagnosed under light microscope and five random field of view were evaluated at × 100 and × 400. Each tumor was given a score according to the intensity of the nuclear and cytoplasmic staining (no staining, 0; weak staining, 1+; moderate staining, 2+; strong staining, 3+; the final score was determined by the multiplying the staining intensity and percentage of the stained cells [14].

Statistical analysis

All morphometric data were collected blindly. Statistical significance for comparison between two measurements was determined using the unpaired 2-tailed Student's *t* test. One-way ANOVA was used for evaluation of the three measurements. Values of $p < 0.05$ were considered significant. GraphPad Prism 7 (GraphPad Software, Inc. La Jolla, CA, USA) was used for statistical analysis.

Results

Expression of CAS in normal testes and human testicular germ cell tumors

We performed immunohistochemistry using a tissue array composed of 102 samples including five human normal testes tissues and different types of human testicular germ cell tumors. CAS-positive staining was detected in the normal testicular tissues, cancer adjacent normal testicular tissues, seminoma, yolk sac tumor, and teratoma. Only two samples were negative. The positive rate of CAS staining in the tissue array was listed in the Supplementary Table 1.

As shown in Fig. 1, CAS in normal testicular tissues and cancer adjacent normal tissues were mainly localized in the nuclear of the germ cells (upper two panels), particularly in the spermatocytes (red arrowhead) but not on the spermatozoa (green arrowhead). Furthermore, the expression of

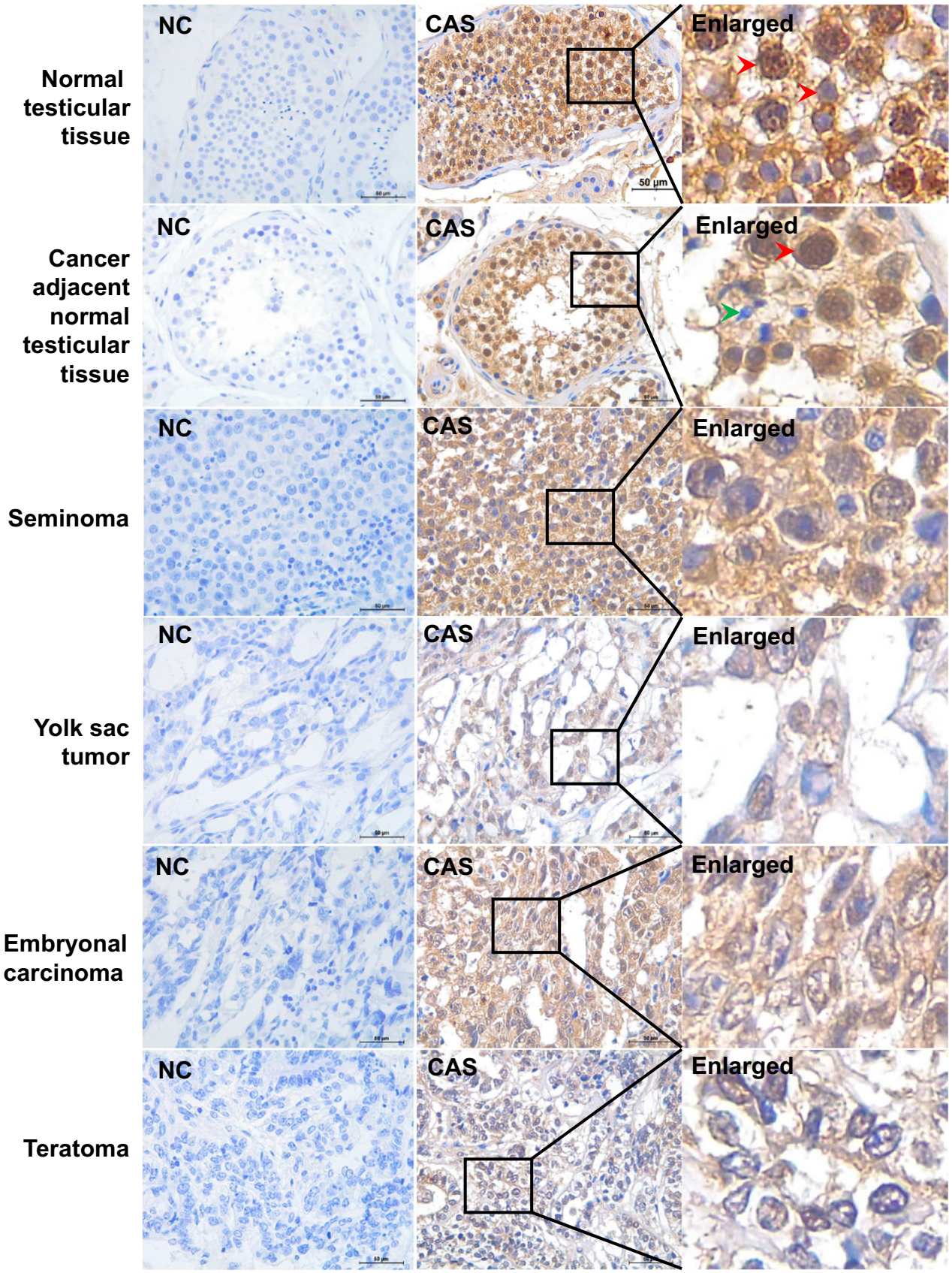
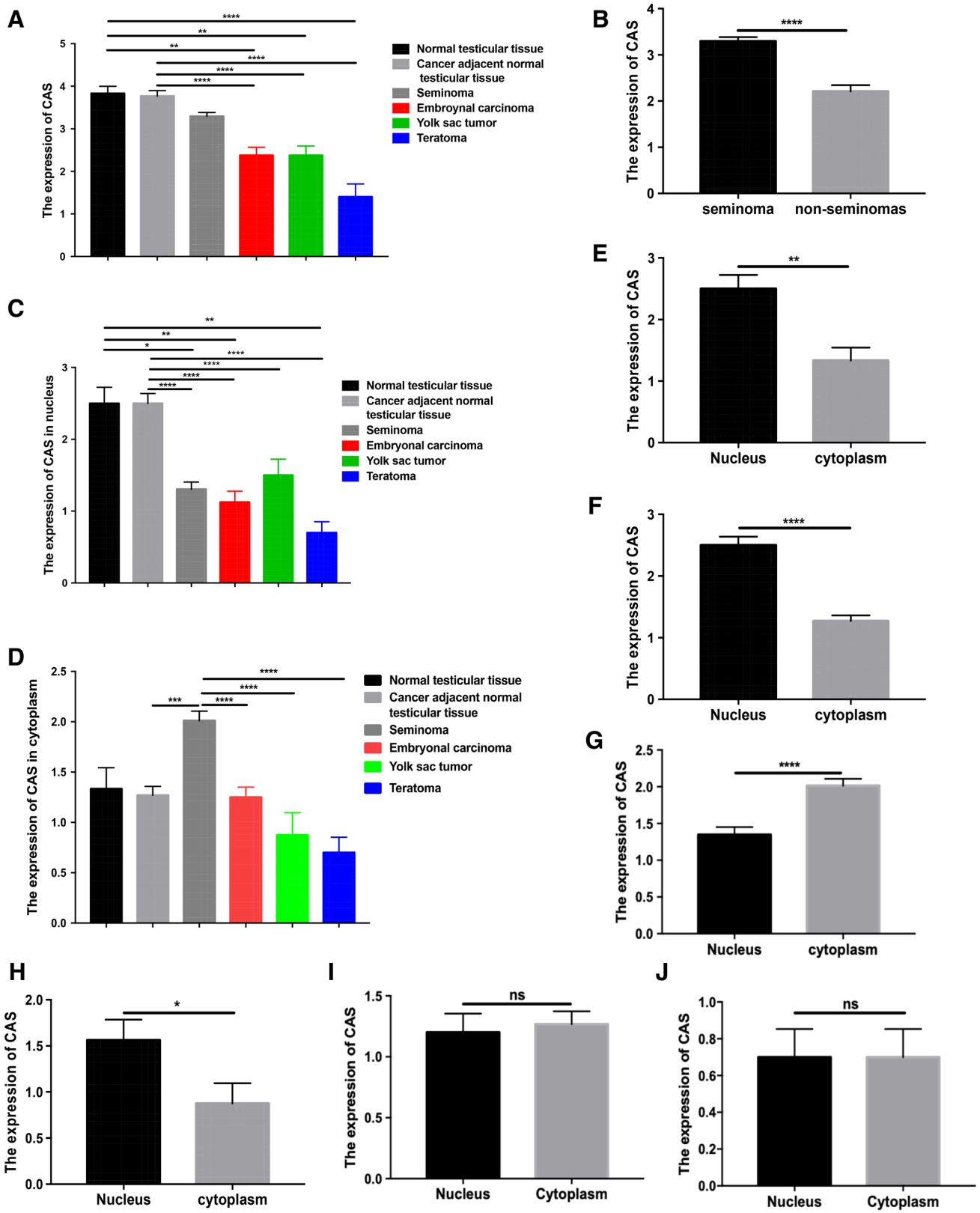


Fig. 1 Representative immunohistochemical images of CAS/CSE1L staining in testicular tissues. Strong staining of CAS was found to be spermatocytes and round spermatids (red arrowhead). *NC* negative control, *CAS* CAS staining. Scale bars, 50 μm



CAS was obviously decreased in non-seminoma samples compared with the normal tissues (Figs. 1 and 2a). Remarkably, the expression of CAS in seminomas was significantly higher than that in non-seminoma (Fig. 2b), which consisted with the data collected from TCGA database (Supplementary Fig. 1).

◂**Fig. 2** Statistical analysis for the expression of CAS in human testicular tissues and comparison of CAS in nucleus and cytoplasm in different human testicular tissues. **a** The corresponding statistical analysis of the CAS intensity in human testicular tissues. **$p < 0.01$; ****$p < 0.0001$. **b** The comparison of CAS intensity between seminoma and non-seminoma. ****$p < 0.0001$. **c** The nuclear CAS in TGCTs was significantly lower than that in normal testicular tissue and cancer adjacent normal testicular tissue. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$. **d** The expression level of CAS in cytoplasm in seminoma was higher than that in normal testicular tissue, cancer adjacent normal testicular tissue, and other germ cell tumors. ***$p < 0.001$; ****$p < 0.0001$. The corresponding statistical analysis of the cytoplasmic CAS intensity in normal human testicular tissues (**e**), cancer adjacent normal testicular tissues (**f**), seminoma (**g**), yolk sac tumor (**h**), embryonal carcinoma (**i**), and teratoma (**j**). *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$

Cytoplasmic translocation of CAS in seminomas

Previous studies have observed that abnormal distribution of CAS contributed to the progression of carcinomas [5, 8, 14]. Therefore, we further compared the location of CAS in the nuclear and cytoplasm separately. As shown in Fig. 2c, more nucleus staining of CAS were detected in normal testicular tissue group and cancer adjacent normal testicular tissue group, while the staining of CAS in the TGCTs was much lower than the normal testicular tissue group. Interestingly, the cytoplasmic CAS in seminoma was dramatically higher than that in normal testicular tissues, cancer adjacent normal testicular tissue, and non-seminomas group (Fig. 2d). In addition, a comparison of CAS in the cytoplasm and nucleus was conducted to reflect the translocation of CAS in different TCGTs. Although no significant difference in the embryonal carcinoma and teratoma (Fig. 2i, j), low expression of CAS in the cytoplasm was found in normal testicular tissues, cancer adjacent normal testicular tissues, and yolk sac tumor (Fig. 2e, f, h). Interestingly, the expression of CAS was significantly elevated in the cytoplasm compared to that in the nucleus in seminoma (Fig. 2g). These results suggested that the translocation of CAS from nuclear to cytoplasm might contribute to the development of seminoma.

Discussion

While upregulation of CAS in breast cancer and colorectal cancer has been observed [4, 9, 16], the expression of CAS in human testis and testicular cancers is still unclear. In the present study, we have revealed the location of CAS in normal human testis. Furthermore, we have demonstrated the elevated cytoplasmic expression of CAS in the seminoma compared with the normal control, while the total expression of CAS was not significantly different between the seminoma and normal testicular tissues. These observations suggest the translocation of CAS plays important role in the progress of seminoma.

The occurrence and development of testicular germ cell tumor are related to multiple oncogenes and cancer suppressor genes. Testicular cancer is the most common malignancy among men between 14 and 44 years old. Although 5-year survival values for TGCTs have risen from < 30% in the 1950s to ~ 95%, the incidence of TGCTs has risen over the past two decades by more than 52,000 new cases annually diagnosed worldwide [1]. Therefore, new effective molecule is still needed for early diagnose and find new therapeutic target.

CAS has been studied and well established as a cell migration and survival regulator during cancer development [7, 17]. Several studies have demonstrated that CAS was associated with regulation of MMP-9 and MMP-2 in melanoma cells and colorectal cancer via interfering the microvesicle generation [3, 16, 18], resulting in the invasiveness of tumor cells. Juliane et al. reported that high expression of CAS was associated with macroangioinvasion in hepatocellular carcinoma (HCC) [6]. It should be noted that our previous study has observed a downregulation of CAS in the lesions of endometriosis; however, high expression of CAS was found to promote the tumorigenesis in some cancers in contrast to a lower expression in TGCTs observed in the present study. The CAS-dependent tumorigenesis involved in the cancers studied and TGCTs appear to be different.

CAS, also named as exportin-2, was demonstrated as the re-export of importin alpha mediated with CAS [19]. Although lots of studies have reported the nuclear location of CAS, translocation of CAS from nucleus to cytoplasm was observed in several cancer cells [5, 8, 9, 14, 16]. A recent study illustrated that depletion of cytoplasmic CAS by siRNA led to the reactivation of endogenous methylated genes, indicating its important role of importin-mediated protein nuclear transportation [20]. Our previous study also demonstrated the role of cytoplasmic CAS in the regulation of EMT during endometriosis progression [13]. Consistent with previous findings, the present results indicated that aberrantly high expression of CAS in cytoplasm, but not in nuclear conferred a propensity of the progression of seminoma. However, the limitation of the present study is that current results could not exclude the possibility of predominant expression of CAS in the spermatogonia cells contributing the tumorigenesis of TGCTs. The detailed mechanisms still need to be further investigated.

In conclusion, our results showed the expression patterns of CAS in normal testicular tissues and TGCTs, indicating that the translocation of CAS might contribute to the progression of seminoma. The present finding, therefore, suggests that targeting CAS may be an attractive alternative for the intervention of TGCTs. The present findings warrant

future investigation of diagnostic and treatment strategies for TGCTs targeting CAS and related signaling.

Author contributions HC and KPG conceived and designed the experiments. JNL, RGH, MY, HZ, XW, and HRG performed the experiments and analyzed the data. YTG and XFL collected the TGCTs. FHL and ZJZ gave intellectual advice and revised the manuscript. JNL and HC wrote the paper.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The project was approved by the Ethic Committee of The Second Hospital of Shanxi Medical University.

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