Original Article
Expression status and prognostic value of cancer/testis antigen OY-TES-1 in glioma

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Abstract: Cancer testis (CT) antigens are attractive therapeutic targets for tumor immunotherapy because of their restrictive expression in normal tissues and excessive in majority of tumor types. OY-TES-1 is a member of the cancer/testis (CT) antigen family. Current research about OY-TES-1 expression in glioma is practically at mRNA level. The role of OY-TES-1 protein in glioma has not yet been described. In this study, we detected OY-TES-1 protein expression in 124 samples from patients with glioma by immunohistochemistry and analyzed the correlation between OY-TES-1 expression and clinical indexes. Furthermore, its clinical significance on glioma prognosis was determined by follow-up data. Our results showed that the OY-TES-1 staining was mainly located in the cell cytoplasm and nucleus and the total positive rate of its protein was 69.35% in 124 isolated tissue samples. OY-TES-1 expression was closely related with the WHO glioma grade. Kaplan-Meier analysis revealed a significant negative correlation between OY-TES-1 expression and survival. OY-TES-1 expression may be a candidate biomarker for prediction of glioma progression and survival of patients.

Keywords: Cancer/testis antigen, OY-TES-1, glioma, prognostic

Introduction
Glioma is the most common central nervous system tumor which accounted for about 50% [1]. Among the different types of gliomas, glioblastoma is one of the most malignant tumors whose median survival time is only about 14 months [2]. Currently, the traditional treatment method is surgery combined with adjuvant radiotherapy and chemotherapy [3]. Unfortunately, it is still failed to significantly prolong the survival of patients with glioma. The main causes are the presence of blood brain barrier making chemotherapy drugs difficultly enter the cranio-cerebral, drug resistance of glioma [4] and diffuse infiltrating of tumor cells giving rise to the low total resection rate of glioma. In addition, the presence of cancer stem cell which acting as the source of tumors lead to high relapse rates and high metastasis rate [5]. Therefore, it is urgent to develop a new adjuvant therapy. Immunotherapy is one of such desirable treatment methods. For many years the central nervous system was believed to have immune privilege [6]. But in recent years, the study found the destruction of blood brain barrier would be happened in some malignant glioma patients. Astrocytes and microglia cells have ability of antigen presentation, thus immunocyte could enter the brain tissues. These pathological changes provide the evidence of practicability of brain tumor immunotherapy [7].

All the immunotherapy are premised on the basis of suitable tumor associated antigen [8]. Cancer testis antigen (CTA) is such a group of tumor antigens because they are almost expressed in majority of tumors and restrictive-ly expressed in normal tissues (except for testis and placenta). It has been proved that a part of CTA could cause the humoral and cellular immune response [9]. These properties render
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them as attractive targets for cancer immuno-
therapy. Currently, tumor immunotherapy tar-
getting some of cancer testis antigens, such as
MAGE-A1, MAGE-A3 and NY-ESO-1 were already
on the stage of clinical trials and got satisfied
results [10-12]. However, there are only a few
relevant reports of CTA expression in glioma at
the present stage [13-15]. The lack of under-
standing of CTA expression in glioma restricts
the further study of CTA application in
immunotherapy.

OY-TES-1, as a member of CTA family, is also
called CT23 according to its ranking in CTA
database (http://www.cta.lncc.br). It was ini-
tially identified as the human homologue of pro-
acrosin binding protein sp32 precursor and first
reported by Ono T in 2001 [16]. In human cells,
OY-TES-1 is located on chromosome 12p12-
p13 and contains ten exons. It has been con-
firmed that OY-TES-1 mRNA was not expressed
in a variety of normal tissues (except for testis)
but detected in multiple tumors tissues (mam-
mary carcinoma, hepatoma, colorectal carci-
noma and epithelial ovarian cancer) with 15% to
40% positive rate [16, 17]. Some tumor cell
lines, such as ovarian cancer cell lines, pros-
tate cancer cell lines, lung cancer cell lines and
breast cancer cell lines were also certified
expressing OY-TES-1 mRNA. In addition,
Tammela et al [17] detected OY-TES-1 protein
expression in different pathological types of
ovarian cancer and found its positive rate of
protein expression was much higher than in
normal tissues. In assay of serum antibody,
about 3% to 10% of cancer patients have pro-
gressed humoral immune response to OY-TES-1
but no corresponding antibody could be detect-
ed in healthy human serum [18]. In the identifi-
cation of antigenic peptides, Okumura [19]
identified HLA-A24 restricted antigen peptide,
which is located at the carboxyl terminus of
OY-TES-1 (TES 401-409). Vitro experiment
results showed that Cytotoxic T lymphocyte
CTL), which induced by dendritic cells (DCs)
loaded with this antigen peptide could specifically kill OY-TES-1 mRNA positive tumor cell
lines. Our previous study had found CTL induced
by OY-TES-1 fusion protein sensitized DC could
effectively attack and kill hepatoma cells as
well. In 2010, Whitehurst [20] found that
OY-TES-1 was both necessary and sufficient for
paclitaxel resistance in ovarian cancer cell
lines and ovarian tumor explants. Moreover,
high OY-TES-1 expression indicated shorter sur-
vival time and faster relapses among ovarian
cancer patients. Recent studies have showed
that OY-TES-1 was related with apoptosis,
migration and invasion of tumor cells [21-23].

All of the findings above suggested that
OY-TES-1 may be used as candidate target gene
for tumor immunotherapy. Our group had con-
formed previously that the expression rate of
OY-TES-1 mRNA in glioma tissues was 80.4%
(41/51) and the protein expression was also
detected in glioma. Besides, normal brain tis-
ues and adjacent non-tumor tissues were not
expressed OY-TES-1. It prompted that OY-TES-1
may be an adaptive target for glioma immuno-
therapy as well. However, our previous study
focus attention on mRNA level but the biologi-
cal effects of gene mainly work on the protein
level. Therefore in our present study, we ana-
yzed OY-TES-1 protein in 124 glioma patients
and further evaluate associations of OY-TES-1
overexpression with clinical indexes (age, sex,
tumor size, WHO grade and KPS) and clinical
outcome by follow-up data. Our results will pro-
vide novel evidences for glioma immunothera-
py possibility.

Table 1. Clinical parameters of patients with
glioma

<table>
<thead>
<tr>
<th>Variable</th>
<th>n = 124</th>
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<tr>
<td></td>
<td>No. (%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>78</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>&lt;38</td>
<td>62</td>
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<tr>
<td>≥38</td>
<td>62</td>
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<td>Tumor size (cm)</td>
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<tr>
<td>&lt;5</td>
<td>54</td>
</tr>
<tr>
<td>≥5</td>
<td>60</td>
</tr>
<tr>
<td>WHO grade</td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>55</td>
</tr>
<tr>
<td>III-IV</td>
<td>69</td>
</tr>
<tr>
<td>KPS</td>
<td></td>
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<td>&lt;70</td>
<td>52</td>
</tr>
<tr>
<td>≥70</td>
<td>61</td>
</tr>
</tbody>
</table>

Abbreviations: WHO grade, 2007 World Health Organiza-
tion Classification of Tumors of the Nervous System;
KPS, Karnofsky Performance Scale; *10 cases of
specimens without records of tumor size; *11 cases of
specimens without KPS score data.
Immunostaining with polyclonal anti-human OY-TES-1 antibody were prepared by our laboratory [26, 27]. Continuous sections in 4 μm thick were prepared from each formalin-fixed, paraffin embedded tissue. Subsequently, the sections were heated in citrate buffer (pH 6.0) for high-temperature antigen retrieval. After endogenous peroxidase had been inactivated by 3% hydrogen peroxide, the sections were immunostained with anti-OY-TES-1 polyclonal antibody (1:200 dilution) or pre-immune serum (negative control) overnight at 4°C. Then the treated sections were recovery at room temperature and incubated with the biotinylated second antibodies (ZSGB-BIO, China). Lastly, immunoreactivity was visualized with 3, 3’-diaminobenzidine (DAB) (Maixin Biotechnology, China) followed by hematoxylin counterstain.

The results were recorded and quantitatively analyzed using the pathological image computer analysis system. We found the highest area with the OY-TES-1-positive cancer cell ratio at low magnification (× 100) and the percentage of positive cells was calculated at high magnification (× 400). According to the staining intensity and the percentage of positive cells, the expression of OY-TES-1 proteins can be analyzed semi-quantitatively. Staining localized to the cell cytoplasm and nucleus was graded on a 0 to 3 intensity scale (0 = no staining; 1 = weak staining; 2 = moderate staining; 3 = strong staining). Positivity was defined as more than 5% of the tumor cells stained by the antibody. According to the sum of both points, the final score of each section was graded on 0 to 6 (0~1 = negative; 2~3 = weakly positive; 4~5 = moderate positive; 6 = strong positive). We utilized the receiver operating characteristic curve analysis to determine the threshold of positive protein expression then stipulated low OY-TES-1 expression when the sum score was between 1 to 3, and high OY-TES-1 expression when the sum score was greater than or equal to 4.

Follow-up

Among 124 patients, there were 84 patients (median 39 years) be tracked by postoperative follow-up and telephone interview (Table 2). Follow-up period was defined from hospital dis-
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OY-TES-1 mRNA in astrocytoma, glioblastoma, mixed glioma and oligodendrogial tumor tissues were all increased significantly (P<0.05). But the OY-TES-1 proteins were not detected in 23 cases of glioma specimens in Human Protein Atlas database (http://www.proteinatlas.org).

**OY-TES-1 expression in glioma tissue**

We analyzed OY-TES-1 expression in 124 glioma tissues by IHC performed with OY-TES-1-specific antibody. The results showed staining was mainly located in the cell cytoplasm and nucleus and the total positive rate of OY-TES-1 protein was 69.35% (86/124); In low-grade glioma (WHO I-II) and high-grade glioma (WHO III-IV), the positive rate was 21.77% (27/55) and 47.58% (59/69), respectively (Table 3). According to observation of staining intensity, high OY-TES-1 protein expression (Figure 2A and 2B) was demonstrated in 70 of 124 patients (56.45%) and low (Figure 2C) in the other 54 patients (43.55%). Meanwhile, we detected 5 normal brain tissues and 1 normal testis tissue. OY-TES-1 was almost not expressed in normal brain tissues (Figure 2D). Testis germinal epithelium and sperm cell (Figure 2E) showed protein positive reaction. Negative control section was immunostained by pre-immune serum which substitute for anti-OY-TES-1 polyclonal antibody (Figure 2F).

**Association of OY-TES-1 expression with clinical index in glioma**

OY-TES-1 expression was significantly associated with the WHO classification, PS3 and Ki67 expression (P<0.05). However, it was not associated with age, gender, tumor size and KPS score (P>0.05) (Table 3).

**Relationship between OY-TES-1 expression and prognosis in patients with glioma**

To provide a powerful explanation of the prognostic role of OY-TES-1, we assessed the effects...
Prognostic significance of OY-TES-1 expression in glioma

Table 3. Correlation between the OY-TES-1 protein and clinical characteristic of glioma patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Positive/Total test (%)</th>
<th>Positive/total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>55/124 (44.35)</td>
<td>46/124 (37.10)</td>
</tr>
<tr>
<td>Female</td>
<td>31/124 (25.00)</td>
<td>24/124 (19.35)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;39</td>
<td>38/124 (30.65)</td>
<td>31/124 (25.00)</td>
</tr>
<tr>
<td>≥39</td>
<td>48/124 (38.71)</td>
<td>39/124 (31.45)</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>36/124 (29.03)</td>
<td>30/124 (24.19)</td>
</tr>
<tr>
<td>≥5</td>
<td>44/124 (35.48)</td>
<td>34/124 (27.42)</td>
</tr>
<tr>
<td>WHO grade</td>
<td></td>
<td></td>
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<tr>
<td>I-II</td>
<td>27/124 (21.77)</td>
<td>18/124 (14.52)</td>
</tr>
<tr>
<td>III-IV</td>
<td>59/124 (47.58)</td>
<td>52/124 (41.94)</td>
</tr>
<tr>
<td>Ki-67 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10%</td>
<td>40/124 (32.26)</td>
<td>28/124 (22.58)</td>
</tr>
<tr>
<td>≥10%</td>
<td>46/124 (37.10)</td>
<td>42/124 (33.87)</td>
</tr>
<tr>
<td>P53 (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10%</td>
<td>35/124 (28.23)</td>
<td>24/124 (19.35)</td>
</tr>
<tr>
<td>≥10%</td>
<td>51/124 (41.13)</td>
<td>46/124 (37.10)</td>
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<tr>
<td>KPS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;70</td>
<td>40/124 (32.26)</td>
<td>28/124 (22.58)</td>
</tr>
<tr>
<td>≥70</td>
<td>43/124 (34.68)</td>
<td>35/124 (28.23)</td>
</tr>
<tr>
<td>Total</td>
<td>86/124 (69.35)</td>
<td>70/124 (56.45)</td>
</tr>
</tbody>
</table>

Abbreviations: High, OY-TES-1 protein expression (++/++); Low, OY-TES-1 protein expression (+/-); KPS, Karnofsky Performance Scale; ***, \(P<0.001\).

Discussion

Immunotherapy is an attractive adjuvant therapy which is regarded as an important method of anti-tumor therapy after surgical resection and chemoradiotherapy. The aim of tumor immunotherapy is to improve anti-tumor immunity so as to control tumor growth or kill tumor cells. Currently, Strategies for tumor immunotherapy mainly includes tumor vaccines, non-specific immune stimulants, adoptive cellular immunotherapy and so on. Among them, tumor vaccine is one of the hot spots in recent years. Its principle is activation of the immune system by exogenous tumor antigen. According to the use of tumor vaccines, it can be divided into two types: one is prophylactic vaccines, namely preparing tumor-related gene vaccines to inoc-
ulate the susceptible population and finally control tumorigenesis. Another is therapeutic vaccines, these are based on tumor-associated antigen and mainly used for adjuvant therapy after chemotherapy.

No matter which kind of immunotherapy, suitable tumor-associated phase specific antigen is the major premise. We considered that over-expression in tumor tissues and almost no expression in normal tissues was the desirable...
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tumor-associated antigen for immunotherapy. OY-TES-1 is such an ideal tumor-associated antigen which restrictedly expressed in normal tissues and overexpressed in various tumor types [21, 22, 26, 29]. Recent research indicated that it was related with apoptosis, migration and invasion of tumor cells [23]. Currently, the research of OY-TES-1 was mainly focused on the levels of mRNA but the protein level was less reported, Tammela and other researchers [17] detected the positive rate of OY-TES-1 protein expression in different pathological types of ovarian cancer tissues and found clear-cell carcinoma proportion was 75% (3/4), endometroid carcinoma was 100% (1/1) and papillary serous carcinoma was 76% (26/43), but other normal tissues (except for testis) such as brain, heart, lung, skeletal muscle, kidney, ovary and stomach were not detected. Our early study showed OY-TES-1 mRNA was expressed on 80.39% (41/51) of glioma tissues. However, the expression of genes involved in many regulatory mechanisms, therefore, the expression of mRNA cannot completely represent the expression of its protein.

In this experiment, we collected a total of 124 cases of glioma tissues for IHC detection. The expression rate of OY-TES-1 protein was 69.35% (86/124). There was a positive correlation between antigen expression and WHO classification and Ki-67 /P53 expression (P<0.05). Survival analysis with clinical data of patients showed that the prognosis of the high expression of OY-TES-1 protein (+/+///) group is poorer than the protein (-/+) group in patients with glioma. Combined with our previous reports, the positive rate of OY-TES-1 antibody was 15.68% (8/51) in serum of patients with glioma, but the corresponding antibody was not detected in healthy persons. All of the findings above indicated that OY-TES-1 high expressed

Figure 3. Survival analysis in glioma patients based on OY-TES-1 expression. Kaplan-Meier curves for overall survival (OS) according to OY-TES-1 expression in all follow up patients (A), low grade glioma patients (B) and high grade glioma patients (C), respectively. (A) Glioma patients with high OY-TES-1 expression had obviously shorter survival than those with low OY-TES-1 expression (P = 0.003); (B) WHO I-II grade glioma patients with high OY-TES-1 expression had shorter survival than those in same grade with low OY-TES-1 expression (P = 0.024); (C) There was no significant difference between high and low OY-TES-1 expression in WHO III-IV grade glioma patients (P = 0.681).
specifically in glioma but almost not expressed in normal tissues, and it could stimulate the humoral immune response. These suggested that OY-TES-1 may be one of the candidate target genes for immune therapy of glioma.

Comparing with our previous study, we found the positive rate of OY-TES-1 protein was lower than mRNA levels. The reasons of this inconsistent phenomenon might be mainly by three possibilities below. Firstly, perhaps PCR was more sensitive than immunohistochemistry. The second was the protein translation pathway that had an obstacle so leading to parts of mRNA was unable to translate into proteins. The third was antigenic loss occurred during tumor progression potentially.

Our result suggested that preparing OY-TES-1 antigen-related tumor vaccine might enhance therapy effect of glioma in future. However, from the view point of our research or pertinent published literatures, this treatment concept was still in initial stage and needed further discuss. In addition, from our study alone, the quantity of samples was needed to be increased and the classification of glioma patients was needed to balance as well. Moreover, we should detect the mRNA levels of corresponding samples in order to further understand the relationship between OY-TES-1 and its protein expression. Detecting the relevant antibody in the sera of patients was also essential. Our ultimate goal was through induction of cytotoxic T cells in vitro to observe its lethal effect of OY-TES-1 expressive tumor cells, than established a foundation of immune therapy based on OY-TES-1 for the future.

Conclusions

OY-TES-1 protein was expressed in glioma at a high frequency and it was also significantly correlated with glioma grade. The patients with higher OY-TES-1 expression have poorer prognosis than those with low expression of its protein. These results indicate OY-TES-1 is probably a novel therapeutic target for tumor immunotherapy in future.

Acknowledgements

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Disclosure of conflict of interest

None.

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Table 4. Univariate and Multivariate analysis of different prognostic parameters

<table>
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<tr>
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<th>n</th>
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<th></th>
<th>Multivariate</th>
<th></th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>HR (95% CI)</td>
<td>P value</td>
<td>HR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Gender (Male)</td>
<td>56</td>
<td>0.849 (0.470-1.533)</td>
<td>0.587</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Age ≥39 (years)</td>
<td>44</td>
<td>2.564 (1.419-4.630)</td>
<td>0.002**</td>
<td>2.103 (1.138-3.886)</td>
<td>0.018**</td>
</tr>
<tr>
<td>Tumor size (≥5 cm)</td>
<td>45</td>
<td>0.820 (0.465-1.446)</td>
<td>0.492</td>
<td>-</td>
<td>-</td>
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<tr>
<td>KPS score (&lt;70)</td>
<td>33</td>
<td>0.686 (0.381-1.235)</td>
<td>0.209</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WHO grade (III + IV)</td>
<td>45</td>
<td>2.316 (1.286-4.170)</td>
<td>0.005**</td>
<td>1.253 (0.558-2.814)</td>
<td>0.584</td>
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<tr>
<td>Ki-67 expression (≥10%)</td>
<td>36</td>
<td>1.671 (0.957-2.944)</td>
<td>0.071</td>
<td>0.855 (0.434-1.684)</td>
<td>0.650</td>
</tr>
<tr>
<td>p53 expression (≥10%)</td>
<td>44</td>
<td>1.747 (0.984-3.102)</td>
<td>0.057</td>
<td>1.504 (0.815-2.775)</td>
<td>0.191</td>
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<tr>
<td>OY-TES-1 expression (High)</td>
<td>52</td>
<td>2.549 (1.359-4.781)</td>
<td>0.004**</td>
<td>2.035 (1.062-3.899)</td>
<td>0.032**</td>
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Univariate analysis was performed using the log-rank test. Multivariate analysis was performed using the Cox proportional hazards model. Abbreviations: HR, Harzard ratio; 95 percent CI, 95 percent confidence interval for relative risk; *P<0.05; **P<0.01.
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References


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combine with RNAi and oligonucleotide micro-

[23] Fu J, Luo B, Guo WW, Zhang QM, Shi L, Hu QP,
Chen F, Xiao SW and Xie XX. Down-regulation
of cancer/testis antigen OY-TES-1 attenuates
malignant behaviors of hepatocellular carci-
8: 7786-7797.

[24] Fuller GN and Scheithauer BW. The 2007 Re-
vised World Health Organization (WHO) Classi-
fication of Tumours of the Central Nervous Sys-
tem: newly codified entities. Brain Pathol

[25] Louis DN, Ohgaki H, Wiestler OD, Cavenee WK,
Burger PC, Jouvet A, Scheithauer BW and Klei-
hues P. The 2007 WHO classification of tu-
mours of the central nervous system. Acta

[26] Luo B, Yun X, Fan R, Lin YD, He SJ, Zhang QM,
Mo FR, Chen F, Xiao SW and Xie XX. Cancer
testis antigen OY-TES-1 expression and serum
immunogenicity in colorectal cancer: its rela-
tionship to clinicopathological parameters. Int

[27] Fan R, Xiao SW, Huang W, Luo B, Li Q, Xu LX
and Xie XX. Preparation and Identification of
Polyclonal Antibody against Cancer-Testis Anti-
gen OY-TES-1. Chinese Journal of Biologicals
2009; 804-806.

[28] Rhodes DR, Yu J, Shanker K, Deshpande N,
Varambally R, Ghosh D, Barrette T, Pandey A
and Chinnaiyan AM. ONCOMINE: a cancer mi-
croarray database and integrated data-mining

and Xie XX. Cancer testis antigen OY-TES-1:
analysis of protein expression in ovarian can-
cer with tissue microarrays. Eur J Gynaecol On-