Immuohistochemical expression and prognostic significance of PD-L1 and P53 expression in astrocytoma
Marwa S. Abd Allah, Rasha M. Abd Raboo and Shaimaa N. Elzamly

Background Astrocytoma is the most common brain tumor. The aberrant expression of programmed cell death ligands 1 (PD-L1) on tumor cells results in tumor immune evasion with tumor aggressiveness in many cancers.

Materials and methods Immunochemistry was used for examining the morphology and the expression of PD-L1 and P53 in 42 cases of astrocytomas. Univariate analysis was used to correlate the protein expression with the variable clinicopathological parameters. Log-rank test was used to compare the survival among the different groups.

Results PD-L1 positive expression was found in 42.9% (18/42) of patients with astrocytoma. PD-L1 positive expression showed statistically significant association with grade of astrocytoma (P = 0.003), poor survival (P = 0.005), and recurrence (P = 0.001). P53 high expression was found in 47.6% (20/42) of patients with astrocytoma. P53 high expression showed statistically significant association with grade of astrocytoma (P = 0.014), survival (P = 0.032), and recurrence (P = 0.001). High significant association was detected between PD-L1 positivity and P53 high expression (P = 0.005).

Conclusion The high expression of PD-L1 and P53 was associated with higher grade and poor survival in astrocytoma. A significant association between PD-L1 and P53 might emphasize the interaction between P53 and PD-L1 during astrocytoma progression. Egypt J Pathol 38:319–325 © 2018 Egyptian Journal of Pathology.

Keywords: immune evasion and astrocytoma, P53, programmed cell death ligands 1

Introduction
Astrocytomas are considered the most common primary brain tumor in adults, estimated at ~51.4% of all primary brain tumors. Astrocytomas had been classified into low-grade astrocytomas, which include pilocytic astrocytoma (grade I) and diffuse astrocytoma (grade II), in addition to high-grade astrocytomas, which include anaplastic astrocytoma (grade III) and glioblastoma multiforme (GBM) (grade IV) (Xue et al., 2017).

The 5-year overall survival (OS) rate of patients with low-grade glioma is ~85%. However, the survival rate of patients with high-grade glioma is less than 5% with the standard therapies (Han et al., 2016b). Despite significant advances in therapeutic procedures of astrocytomas, the outcomes remain gloomy. Similar to many other tumors, astrocytomas express or secrete several immunosuppressive molecules that regulate immune cell function (Sui et al., 2015).

Programmed cell death ligands 1 (PD-L1) is an immunosuppressor molecule that suppresses the activation of T cells, leading to the progression of tumors (Brusa et al., 2013). Overexpression of PD-L1 in cancers such as gastric cancer, renal carcinoma, hepatocellular cell carcinoma, esophageal cancer, pancreatic cancer, ovarian cancer, and bladder cancer is associated with poor clinical outcomes. In contrast, PD-L1 expression correlates with better clinical outcomes in breast cancer and Merkel cell carcinoma. However, the prognostic value of PD-L1 expression in lung cancer, colorectal cancer, brain tumors, and melanoma remains controversial (Pyo et al., 2017).

Activation of PD-1/PD-L1 signaling serves as a principal mechanism by which tumors evade T-cell immunologic responses. Blocking antibodies that target PD-1 and PD-L1 were approved to have remarkable response rates in patients with cancer who have PD-L1 overexpressing tumors (Jing et al., 2016).

P53 is a nuclear transcription factor with a proapoptotic function. It plays a crucial role in control of cell cycle, apoptosis, and the maintenance of genomic stability (Cole et al., 2016). As more than 50% of human cancers carry loss-of-function mutations in p53 gene, p53 has been considered to be one of the classical type tumor suppressors (Murata et al., 2013).

Some recent studies have highlighted the possible relation between P53 and PD-L1. Therefore, we initiated the present study to evaluate (a) the immuno-histochemical expression of PD-L1 and P53 in astrocytoma, (b) the correlation between PD-L1 and P53, and (c) the correlation of both PD-L1 and P53 with other prognostic factors and survival.

Materials and methods
This is a retrospective study carried out on 42 cases of astrocytomas. The material included archived formalin-fixed,
paraffin-embedded blocks of 42 astrocytoma cases collected from Pathology Department, Faculty of Medicine, Benha University, between the years 2010 and 2015. Clinicopathological data concerning age, grade, 3-year survival, and recurrence were extracted from the pathology reports and medical records after approval by the institutional review board.

**Histopathological study**
Four-micron-thick sections were stained by conventional hematoxylin and eosin stain. Two blind expert pathologists independently confirmed the morphology and grading of astrocytoma.

**Immunohistochemical study**
Four-micron tissue sections were prepared and immunostained for PD-L1 rabbit polyclonal antibody (Thermo Fisher Scientific, California, Fremont, USA), prediluted ready to use, and P53, Rabbit polyclonal antibody (Thermo Fisher Scientific), prediluted ready to use. The immunohistochemical assay was applied using the standard streptavidin-biotin technique following the manufacturer’s instructions. For secondary reagents, a labeled streptavidin-biotin kit (Neomarker; Lab Vision, USA) has been used. DAB (3,3′-Diaminobenzidine) was used as a chromogen. Human tonsillar tissue was used as a positive control for PD-L1 and breast carcinoma for P53. Negative control for all markers was achieved by omitting the primary antibody.

**Interpretation of immunohistochemical staining.**

**PD-L1**
The expression was designated as cytoplasmic or membranous staining. Tumors with up to 5% of stained cells were considered positive (Zeng et al., 2016).

**P53**
The expression was designated as nuclear staining. P53 immunoreactivity was graded as low expression (<20%) or high expression (>20%) of stained positive cells (Al-Khafaji and Mahmood, 2014).

**Statistical analysis**
SPSS, version 16, software (SPSS Inc., Chicago, Illinois, USA) was used. The collected data were tabulated and analyzed using χ²-test or Fisher’s exact test. The receiver operating characteristics curve analysis was performed to detect the cutoff values for PD-L1 and P53 with optimum sensitivity and specificity in diagnosis of high-grade astrocytomas (Anaplastic-GBM) from low-grade astrocytomas. Log-rank test was used to compare survival between study groups. The accepted level of significance in this work was stated at 0.05 (P<0.05 was considered significant).

**Results**
The present retrospective study enrolled 42 cases of astrocytoma. The age of participants ranged from 2 years up to 73 years. All clinicopathological characteristics were demonstrated in Table 1.

**Immunohistochemical results of PD-L1 and P53**
PD-L1 positive expression was found in 42.9% (18/42) of patients with astrocytoma (Fig. 1). PD-L1 positive expression showed statistically significant association with histopathologic grade of astrocytoma (P=0.003), poor survival (P=0.005), and recurrence (P=0.001) as detailed in Table 2.

The present study showed that positive PD-L1 expression was detected in 69.4% of astrocytoma tissues. All pilocytic astrocytoma cases were negative for PD-L1, whereas PD-L1 positive expression was 33.3% in diffuse astrocytoma (grade 2), 45.5% in anaplastic astrocytoma (grade 3), and 69.2% in GBM (grade 4), and this difference in expression was highly significant among different grades of astrocytoma (P<0.01).

P53 high expression was found in 47.6% (20/42) of patients with astrocytoma (Fig. 2). P53 high expression

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinicopathological features of astrocytoma cases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histopathological parameters</strong></td>
<td><strong>Pilocytic</strong></td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>10.3±6.0</td>
</tr>
<tr>
<td>Range</td>
<td>2–18</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24 (57.1)</td>
</tr>
<tr>
<td>Female</td>
<td>18 (42.9)</td>
</tr>
<tr>
<td>Tumor location</td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>5 (11.9)</td>
</tr>
<tr>
<td>Frontal</td>
<td>10 (23.8)</td>
</tr>
<tr>
<td>Parietal</td>
<td>13 (31.0)</td>
</tr>
<tr>
<td>Frontoparietal</td>
<td>6 (14.3)</td>
</tr>
<tr>
<td>Temporal</td>
<td>8 (19.0)</td>
</tr>
<tr>
<td>Survival</td>
<td></td>
</tr>
<tr>
<td>&lt;3 years</td>
<td>20 (47.6)</td>
</tr>
<tr>
<td>≥3 years</td>
<td>22 (52.4)</td>
</tr>
<tr>
<td>Recurrence</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>27 (64.3)</td>
</tr>
<tr>
<td>Positive</td>
<td>15 (35.7)</td>
</tr>
<tr>
<td>Total</td>
<td>42 (100)</td>
</tr>
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</table>

GBM, glioblastoma multiforme.
showed statistically significant association with histopathologic grade of astrocytoma ($P=0.014$), survival ($P=0.032$), and recurrence ($P=0.001$), as detailed in Table 2.

This current study showed that P53 high expression was detected in 47.6% of astrocytoma with a significant difference in its expression among different histopathologic grades ($P<0.05$), as P53 high expression was 16.7% in pilocytic astrocytoma (grade 1), 33.3% in diffuse astrocytoma (grade 2), 54.5% in anaplastic astrocytoma (grade 3), and 69.2% in GBM (grade 4).

None of the markers were associated with the patient’s age, sex, or tumor location.

**Correlation between PD-L1 and P53**

The present study demonstrated a high significant positive correlation ($P=0.005$) between PD-L1 and P53 immunoreactivity in astrocytoma, as among 20 cases
that showed high P53 expression, 13 (65.0%) cases were positive for PD-L1, as shown in Table 3.

Receiver operating characteristic curves
The receiver operating characteristic curve analysis showed that the diagnostic performance of both PD-L1 and P53 in discriminating high-grade astrocytomas from low-grade astrocytomas was accepted. For PD-L1, cutoff value was 2.5 and area under curve was 0.821. For P53, cutoff value was 6.5 and area under curve was 0.804, as shown in Table 4 and Fig. 3.

Kaplan–Meier analysis results
The OS probability of cases with negative PD-L1 and low P53 was significantly higher than cases with positive PD-L1 and high P53 ($P=0.005$, 0.032, respectively), as shown in Fig. 4.

Discussion
Growing evidence suggests that cancer immune evasion is a hallmark for the development and progression of cancer. Tumors use multiple mechanisms to avoid recognition by the host immune system. PD-L1 expression is an immune evasion mechanism exploited by various malignancies. PD-L1 binds PD-1 to attenuate the cellular immune response by inducing T-cell apoptosis, reduction of proliferation, and inhibition of cytokine secretion (Li et al., 2017).

PD-L1 is not expressed on normal epithelial tissues but is expressed on many cancers including colorectal cancer (Zhao et al., 2014), gastric cancer (Böger et al., 2016), cervical carcinoma (Dong et al., 2018), hepatocellular carcinoma (Hu et al., 2018), and non-small cell lung cancer (Silva et al., 2018).

In the present study, we explored the prognostic significance of PD-L1 and P53 expression by the IHC evaluation in 42 Egyptian patients with astrocytomas of different grades. The relation between both markers was also evaluated.

The present study showed that positive PD-L1 expression was detected in 42.9% of astrocytoma with high significant differences among different grades, being highest in GBM (grade IV) and then anaplastic astrocytoma, and the lowest expression was seen in diffuse astrocytoma, whereas pilocytic astrocytoma cases were completely negative for PD-L1. Our findings suggest that PD-L1 protein expression can contribute to tumor aggressiveness in astrocytoma.

These findings are matching previous studies, such as Garber et al. (2016) who found that PD-L1 positive expression was significantly correlated with tumor grade, with all PD-L1 positive cases being associated with grade IV gliomas. However, Zeng et al. (2016) found no significant association between PD-L1 expression and pathological grade in patients with gliomas. This difference in results may be referred to that antibodies used in different studies have different sensitivity and that expression of PD-L1 in tumors is not uniform, and sampling time and may affect the results of staining.
Table 4  Diagnostic performance for programmed cell death ligands 1 and P53

<table>
<thead>
<tr>
<th>Variables (n = 42)</th>
<th>Cutoff point</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-L1 score</td>
<td>2.5</td>
<td>79.2</td>
<td>66.7</td>
<td>0.821</td>
<td>76.0</td>
<td>70.6</td>
<td>73.8</td>
<td>0.003**</td>
</tr>
<tr>
<td>P53 score</td>
<td>6.5</td>
<td>70.8</td>
<td>72.2</td>
<td>0.804</td>
<td>77.3</td>
<td>65.0</td>
<td>71.4</td>
<td>0.006**</td>
</tr>
</tbody>
</table>

**Highly significant.

AUC, area under curve; NPV, negative predictive value; PD-L1, programmed cell death ligands 1; PPV, positive predictive value.

Fig. 3

The receiver operating characteristics curve for the validity of PD-L1 and P53 to predict high-grade astrocytoma group from low-grade astrocytoma group (a) PD-L1 with AUC of 0.821; (b) P53 with AUC of 0.804. AUC, area under the curve; PD-L1, programmed cell death ligands 1.

Fig. 4

Kaplan–Meier curve for survival according to PD-L1 expression and P53. PD-L1, programmed cell death ligands 1.
The study of Parsa et al. (2007) explained that loss of phosphatase and tensin homolog (PTEN), a major GBM tumor suppressor, induces the expression of PD-L1 and that PTEN loss regulates PD-L1 post-transcriptionally.

In the present study, PD-L1 positive expression was significantly associated with recurrence and poor survival. This matches the studies of Zeng et al. (2016) and Han et al. (2016a). However, Berghoff et al. (2015) found that PD-L1 positive expression was not associated with survival time in GBM.

Several studies have provided evidence of a relationship between shortened survival and PD-L1 high expression as reported by Wang et al. (2016) in gastric cancer, Calderaro et al. (2016) in hepatocellular carcinoma, and Zhou et al. (2017) in non-small cell lung carcinoma. Moreover, a recent meta-analysis study published by Wang et al. (2017) declared that PD-L1 overexpression is significantly associated with poor OS and disease-free survival in many solid tumors, especially in renal carcinoma and urothelial cancer.

PD-L1 was found to be expressed in both tumor cells and antigen-presenting cells. PD-L1 expression on tumor cells provides protection from (CD8 + ) cytotoxic T-cell-mediated cell killing (Juneja et al., 2017). The binding of PD-L1 with PD-1 of T-cell leads to T-cell dysfunction and exhaustion with interleukin-10 production in a tumor mass. Dysfunction of cytotoxic T cells subsequently results in that tumor cells become aggressive and secrete numerous proinflammatory cytokines such as tumor necrosis factor-α (Alsaab et al., 2017).

This current study showed that P53 high expression was detected in 47.6% of astrocytoma cases with significant differences among different grades, being highest in high-grade astrocyomas and the lowest expression was seen in low-grade astrocyomas. High p53 expression showed significant association with recurrence and poor survival.

These findings are consistent with previous studies as Milinkovic et al. (2012) and Al-Khafaji and Mahmood (2014) who declared that high p53 expression was associated with high-grade astrocytoma.

Many previous studies have demonstrated that p53 is overexpressed in most of types of cancer which leads to transcriptional regulation dysfunction and uncontrolled cell growth (Labuschagne et al., 2018).

The present study showed a high significant positive association between PD-L1 expression and P53 expression (P = 0.005), suggesting a link between PD-L1 and p53. To our best knowledge, this is the first study to examine the correlation between PD-L1 and P53 in astrocytoma.

Previous studies have also provided direct relation between PD-L1 expression and P53 status in other cancers, such as the studies of Kan and Dong (2015) in hepatocellular carcinoma, Xu and Zhang (2018) in lung adenocarcinoma, and Yu et al. (2018) in pulmonary lymphoepithelioma-like carcinoma.

Increasing evidence has approved that P53 plays a potential role in regulation of PD-L1 and immune evasion. However, the underlying mechanism for this relation remains unclear. Cortez et al. (2015) demonstrated in a comprehensive study that p53 regulated PD-L1 status via miR-34, which directly binds to the PD-L1 3′ untranslated region in models of NSCLC.

The present work suggests that further studies are needed to declare the interaction between PD-L1 and P53 in astrocytoma to clarify the role of P53 in regulation of PD-L1 and immune evasion.

Conclusion

PD-L1 and P53 were unregulated in high-grade astrocytoma, and their aberrant expression is associated with poor prognostic features, suggesting their potential role in progression of astrocytoma. High PD-L1 expression may be a prognostic indicator for reduced OS. A significant association between PD-L1 and P53 may emphasize the interaction between PD-L1 and P53.

Conflicts of interest

There are no conflicts of interest.

References


