Correlation between Programmed Death-1 Ligand-1 and p53 in Patients with Lung Adenocarcinoma

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Cancer immunotherapy is causing a paradigm shift in the way cancer researchers think about treating people with the disease.[1] Unlike traditional therapies, immunotherapy harnesses the immune system to fight cancer instead of targeting the tumor itself. Lung adenocarcinoma is frequently diagnosed in advanced stages with poor prognosis and has become the most common tumor treated by immunotherapy.

Among all immune checkpoints, programmed death-1 (PD-1) and its ligand PD-1 ligand-1 (PD-L1) have attracted attention globally. The binding of PD-L1 to PD-1 will transfer inhibitory signals to suppress T cell functions so that the tumor cells (TCs) can protect themselves from immune destruction.[2] Blockage of interactions between PD-L1 and PD-1 can reinvigorate T cells into gaining their effector functions of killing TCs. At the same time, drugs (such as nivolumab and pembrolizumab) targeting PD-1 and PD-L1 have exhibited some therapeutic effects.[3]

On the other hand, *p53* is the most frequently studied molecular mutation in human cancer, and p53 protein expression has been found in more than 50% of human tumors. The *p53* is a tumor suppressor gene, which plays a negative regulatory role in cell proliferation and differentiation,[4,5] thus suggesting that aberrant *p53* expression is a prerequisite for tumor development.

Both PD-L1 and p53 play important roles in tumor development. Accordingly, some studies including ours, have found that PD-L1 expression is significantly correlated with p53 status in lung adenocarcinoma.[6,7] Here, we discuss the correlation between *PD-L1* and *p53* expression levels in lung adenocarcinoma and describe the immunohistochemical evaluation of PD-L1 and p53 in our clinical work.

For the mechanism of PD-L1 regulation by p53, the current understanding is that PD-L1 expression could be induced by adoptive immune resistance (secondary to development of multiple passenger mutations leading to cytotoxic lymphocyte/ Th1 cell (CTL/Th1) pathway activation in the tumor)

and innate immune resistance (secondary to oncogenic signaling) and multiple other mechanisms.[8] Since *p53* alterations could be seen in malignant neoplasm, especially in poorly differentiated tumors, it is likely that *p53* is mutated as a part of passenger mutations and *p53* alterations could be a marker for adaptive immune resistance.

Increasing evidence has indicated that *p53* plays a crucial role in the regulation of immune response. As described, TCs lacking wild-type or expressing mutant *p53* had lower levels of transporter associated with antigen processing 1 (TAP1), endoplasmic reticulum aminopeptidase 1 (ERAP), Fas/APO-1, and miR-34 [Figure 1].[9] Among them, lower levels of TAP1 and lower concentration of ERAP1 resulted in limited expression of major histocompatibility complex I on TCs; subsequently, cytotoxic lymphocytes (CTLs) could inhibit the function of killing TCs and identify foreign proteins;[10] downregulation of Fas/APO-1 expression in TCs resulted in the reduction of CTL-mediated apoptosis on the binding of Fas-ligand (FasL) to Fas/APO-1.[11] Low levels of miR34 suppressed relative immune evasion of p53/miR-34/PD-L1 [Figure 1].

Previous research has found that members of the miR-34 family play important roles in immune cell (IC) regulation and act as a bridge between PD-L1 and p53. Members of the MiR-34 family are well-characterized effector molecules that are transcriptionally induced by p53, and they were expressed at elevated levels in cells that expressed wild-type *p53* relative to their controls.[12] On this basis, Cortez *et al*.[13] demonstrated that PD-L1 is regulated by p53 at the molecular level. Briefly, three *in vitro* systems, including the p53+/+

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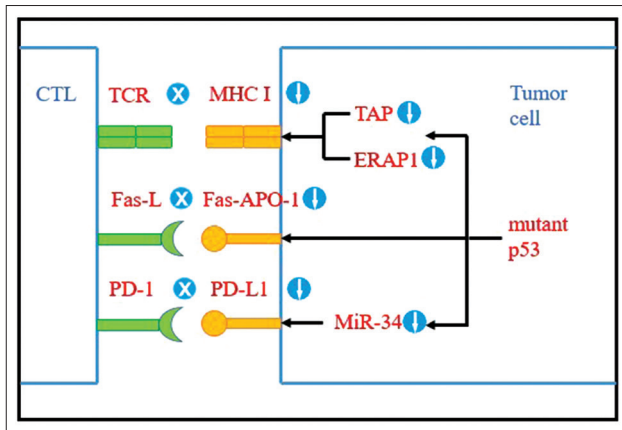| Access this article online	
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Figure 1: Mutant p53 regulated immune response by ERAP1, Fas/APO-1, and miR-34. CTL: Cytotoxic lymphocyte; TCR: T cell receptor; PD-1: Programmed cell death-1; PD-L1: Programmed cell death-ligand 1; MHC I: Major histocompatibility complex; TAP: Transporter associated with antigen processing; ERAP 1: Endoplasmic reticulum amino peptidase 1.

and p53−/− HCT116 cells (a p53-inducible H1299 cell line) treated with nutlin-3 and H460 lung cancer cells with or without knockdown of wild-type *p53*, showed that PD-L1 expression was downregulated by p53 through upregulation of miR-34, which directly binds to the PD-L1 3' untranslated region in models of non-small cell lung carcinoma (NSCLC). PD-L1 was lost or expressed at reduced levels in cells that expressed wild-type *p53*, and tumors with mutated *p53* had low miR-34a and high PD-L1 levels. Therefore, the p53/miR-34/PD-L1 axis may be a novel mechanism of tumor immune evasion.[13]

The correlation between PD-L1 and p53 in lung adenocarcinoma is poorly understood. A small number of studies clarified their correlation at mRNA and immunohistochemistry expression levels. For the mRNA expression level, Cortez *et al.*[13] compared the mRNA expression levels of *p53* and *PD-L1*, and found a statistically significant inverse correlation between them ($P < 0.001$). They also compared *PD-L1* expression in NSCLC tumors with mutated *p53* versus wild-type *p53* and revealed that mutated *p53* tumors had statistically significantly higher *PD-L1* levels than wild-type *p53* tumors ($P = 0.03$). For the immunohistochemistry expression level, Cha *et al.*[6] examined PD-L1 and p53 expression in a total of 323 surgically resected lung adenocarcinoma cases using anti-PD-L1 (clone SP142) and anti-p53 (clone DO-7) antibodies, and found that PD-L1 expression was positive in 18.6% of TCs and 23.5% of tumor-infiltrating ICs, whereas aberrant p53 expression was observed in 33.1% of TCs. Their study demonstrated that PD-L1 positive tumors were significantly associated with p53 aberrant expression ($P < 0.001$).[6] However, no significant association was found between p53 and PD-L1 expression in a group of Egyptian patients with NSCLCs.[14] On this basis, our team also demonstrated that aberrant p53 expression was significantly associated with PD-L1 positivity in

TCs ($P < 0.001$) and tumor-infiltrating ICs ($P = 0.001$) in 229 surgically resected Chinese lung adenocarcinoma patients, and the whole results have been published earlier this year.[7]

It was also found that PD-L1 positive was associated with larger tumors, node metastasis, solid predominant tumors, and poor differentiation; p53 aberrant expression was associated with node metastasis, solid predominant tumors, and poor differentiation. The relevant factors of both PD-L1 and p53 would admittedly predict poor development of tumors.[6,7] The similar conclusion was shown in different groups with a large sample, so we believe p53 was involved in PD-L1 relative tumor immune evasion. The molecular mechanism mentioned above was verified, and there are still some molecular pathways of tumor immune evasion other than p53/miR-34/PD-L1.

To investigate the correlation between PD-L1 and p53, the "positivity standard" should be defined first. In the above studies, PD-L1 expression in TCs was considered "positive" if 5% of TCs (or higher) showed membranous staining. Otherwise, staining was considered negative. PD-L1 expression in tumor-infiltrating ICs was considered positive if 1% of ICs (or higher) showed membranous or cytoplasmic staining, as described previously.[3,6,15,16]

However, there are various PD-L1 immunohistochemical antibodies, such as 22C3, 28-8, SP142, and SP263, of which SP142 is the most commonly used antibody in China. Both TCs and tumor-infiltrating ICs should be evaluated. Recently, Roche established the latest diagnostic criteria on VENTANA PD-L1 (SP142) Assay. Here, we should interpret these criteria in detail due to its significance in PD-L1 testing. The observation process was divided into two steps – Step 1: PD-L1 expression was considered "≥50% TC" if 50% of TCs (or higher) showed positive staining. Otherwise, step 2 was followed. Step 2: PD-L1 expression was considered "≥D10% IC" if 10% of tumor-infiltrating ICs (or higher) showed positive staining. Otherwise, it was considered "<50% TC and <10% IC."

The p53 expression was defined as "aberrant expression" if TCs showed either nuclear expression in greater than 50% or complete absence of staining and as "wild type expression" if TCs showed no aberrant expression (1–50% staining), as references described in lung and ovarian cancer,[6,17,18] which was recommended by our team, while in individual study, p53 expression was evaluated as follows p53-negative (m5%), low p53 (5–50%), and high p53 (>50%).[14]

Some studies also explored the correlation between PD-L1 and p53 in other tumors and revealed several novel insights. For the hepatocellular carcinoma, Kan and Dong[19] detected PD-L1, APE1, and p53 in 128 patients with hepatocellular carcinoma. They showed that the rate of positive PD-L1 expression was 82.03% and the rate of positive aberrant p53 expression was 60.94% in the TCs of hepatocellular carcinoma. Unlike that in lung adenocarcinoma, p53, and PD-L1 expression were inversely correlated ($P = 0.010$).

For the melanoma, PD-L1 positivity was seen in 21% of desmoplastic melanoma patients, and it was significantly correlated with p53 expression ($P = 0.018$). It was also correlated with mixed histology, tumor thickness, and Ki-67 proliferation index, which are signs of tumor aggressiveness and progression.[20] On the other hand, this study also proposed a new hypothesis for the mechanism of PD-L1 regulation by p53, which suggested that ultraviolet light might account for the higher frequency of PD-L1 expression through the p53 pathway in melanoma TCs. The basis for supporting this hypothesis is that melanomas are dependent on ultraviolet-induced DNA damage and that the *p53* gene harbors the most ultraviolet-induced mutations in melanoma.[21] As there was a significant correlation between PD-L1 and p53 expression, upregulation of PD-L1 in melanoma TCs could be partly due to oncogenic pathway activation by ultraviolet light through p53 activity.

PD-1/PD-L1 inhibitors (such as nivolumab, pembrolizumab, and pidilizumab) are considered to have a good safety, little toxicity, and relatively well-tolerated therapeutic method.[2] Thus, we anticipate that they will take revolutionary progress in cancer treatment. As described above, PD-L1 expression is significantly correlated with p53 status at mRNA and protein levels in lung adenocarcinoma, as well as in some other tumors. The correlation between PD-L1 and p53 ties tumor immune evasion to p53-relevant tumor suppressor pathways. Aberrant p53 expression may be useful in lung cancer diagnosis and qualification in anti-PD-L1 therapy and could be a potential target of combination therapy.

Previous survival analysis demonstrated that both PD-L1 and p53 were associated with poor prognosis.[6,7] Thus, more studies will be required to confirm whether the concomitant status of p53 and PD-L1 expression are useful biomarkers of response to immune therapy. Cortez *et al.*[13] found that NSCLC patients expressing high PD-L1 and low p53 levels had lower survival rates than patients with low PD-L1 and high p53 levels. However, it should be noted that this analysis was limited because only mRNA levels and not the protein levels of *p53* and *PD-L1* were available.

We also described the evaluation of PD-L1 and p53 expression in our clinical work, and this was the key point for PD-L1 or p53-relative therapy. However, the diagnostic kit for immunotherapy has not been established. It will be necessary to establish the diagnostic criteria required to predict responses to immune checkpoint inhibitors in further studies. It is known that p53 is not the only factor that contributes to PD-L1 regulation. For instance, PD-L1 expression is strictly associated with microRNA function in lung cancer cells and the group of microRNAs related to PD-L1 includes miR-200, miR-197, and miRNA-34;[22] Epidermal growth factor receptor is also involved in the regulation of PD-L1 expression and cell proliferation through the interleukin (IL)-6/Janus kinase/signal transducers and activators of transcription 3 signaling pathway in NSCLC;[23] Wang *et al.*[24] found that IL-17 and tumor necrosis factor-α act individually, rather than cooperatively, through activation of nuclear factor kappa B and extracellular regulated protein kinases 1/2 (ERK1/2) signaling to up-regulate PD-L1 expression in human prostate and colon cancer cells.[24] More studies on PD-L1 regulation factors are needed before they could be used in the clinic.

In conclusion, p53 plays an important role in PD-L1 regulation, and the concomitant expression of p53 and PD-L1 could be a potential target for combination therapy. To offer personalized immunotherapy to different patients, p53 and PD-L1 status should be taken into consideration.

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Conflicts of interest
There are no conflicts of interest.

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