Lung Cancer 110 (2017) 63–67

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

MYC expression correlates with PD-L1 expression in non-small cell lung cancer

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ARTICLE INFO

Keywords:
Immune checkpoint inhibitors
Myc
Non-small cell lung cancer (NSCLC)
PD-L1

ABSTRACT

Objectives Programmed death-ligand 1 (PD-L1) is a widely used biomarker for predicting immune checkpoint inhibitors, but is of limited usefulness in the prediction of drug response. MYC, a transcription factor that is overexpressed in cancers, is involved in preventing immune cells from attacking tumor cells through inducing PD-L1 expression. This study evaluated the relationship between MYC and PD-L1 expression in 84 non-small cell lung cancer (NSCLC) patients who underwent curative surgical resection.

Materials and Methods The relationship between MYC and PD-L1 was investigated by introducing pcDNA3-cMYC into A549 and H1299 cells with low PD-L1 expression and *si*RNA against MYC into H60 and H2009 cells with high PD-L1 expression. Expression of PD-L1 in NSCLC tissues was analyzed by immunostaining using a PD-L1 (22C3) PharmDx protocol using the Dako Automated Link 48 platform and expression of MYC was determined using anti-c-MYC (Y69) (ab320720).

Results Of 84 patients, PD-L1 was expressed in 14 (16.7%) and MYC was overexpressed in 30 (35.7%). We investigated the relationship between PD-L1 and MYC expression. There were 49 (58.3%) double-negative patients and 9 (10.7%) double-positive patients. Significant positive correlation was observed between PD-L1 and MYC expression ($\gamma = 0.210$, $P = 0.029$). Double-negative patients showed better disease free (31.1 vs. 7.1 months, $P = 0.011$) and overall survival (56.1 vs. 14.4 months, $P = 0.032$) than double-positive patients.

Conclusion Taken together, MYC expression significantly correlated with PD-L1 expression in NSCLC. The usefulness of MYC expression as a surrogate marker of treatment response assessment is worth evaluating for immune checkpoint inhibitor therapy and special interest are required for the subgroup of NSCLC patients, whose tumor expresses PD-L1 and MYC double positive.

1. Introduction

Lung cancer is a major public health issue worldwide, with a high prevalence and high mortality [1]. Throughout the era of platinum-based doublet chemotherapy, target agents have been widely used [2]. However, in cases where a treatment target cannot be identified and patients have resistance to the target agent, new breakthroughs are needed.

The tumor microenvironment interacts with cancer cells by modulating programmed death-1 receptor −1 (PD-1) and the ligand PD-L1 pathways. Expression of PD-L1 on cancer cells leads to evasion from the immune response, permitting cancer progression and metastasis [3,4]. Immunotherapy for lung cancer had entered a golden era with the development of immune checkpoint inhibitors that interfere with cytotoxic T lymphocyte antigen-4 (CTLA-4), PD-1 and its ligand PD-L1. A number of immune checkpoint inhibitors are under development based on biological findings. The PD-1 inhibitors nivolumab and pembrolizumab have received FDA approval for lung cancer treatment [5,7].

Demand for biomarkers for predicting therapeutic outcomes of these drugs is high. Biomarkers developed to date [5] are: (1) PD-L1 expression in cancer cells; (2) tumor microenvironment/immune effector cells (with PD-L1 expression in the tumor microenvironment, PD-L2 expression in tumor cells and infiltrating immune cells, CD8 and T-cell and effector functional markers, and gene expression of CTLA4 and CX3CL1); (3) gene alteration and phenotypic alteration of tumor cells such as tumor mutational load, oncogene mutation, and epithelial mesenchymal transition; and (4) clinicopathological biomarkers such as squamous cell histology and smoking history. In lung cancer, PD-L1 is a therapeutic target of immune checkpoint inhibitors and is used as a biomarker to predict therapeutic response. However, its usefulness as a therapeutic biomarker is challenged because of: (1) differences in antibodies and staining platforms from different companies; (2) instability of PD-L1 antigenicity; and (3) different reading criteria among

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http://dx.doi.org/10.1016/j.lungcan.2017.06.006
Received 6 January 2017; Received in revised form 15 May 2017; Accepted 8 June 2017

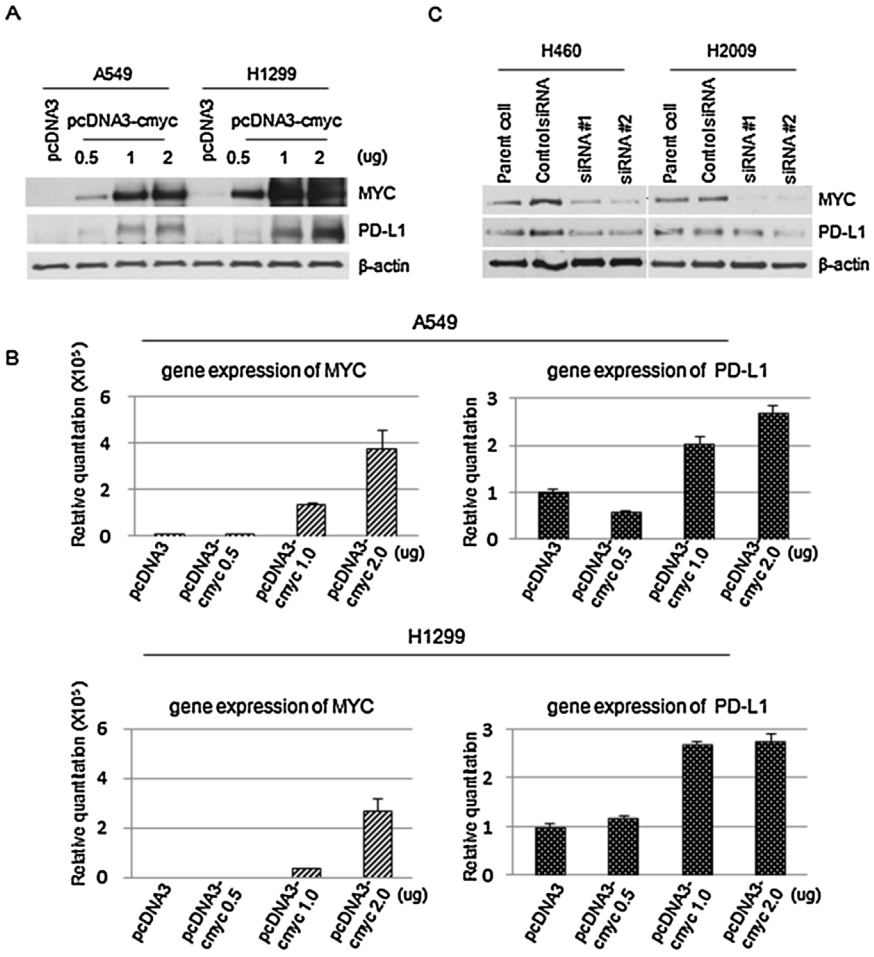

Fig. 1. MYC regulates expression of PD-L1. A549 and H1299 NSCLC cells were transfected with either pcDNA3 (control) or pcDNA3-cMYC and expression of PD-L1 was evaluated by immunoblotting (A) and real-time PCR (B). (C) H460 and H2009 cells were transfected with siRNA against MYC and expression of MYC was evaluated by immunoblotting.

researchers for predicting therapeutic responses. These issues suggest an urgent need for effective surrogate markers for predicting response to immune checkpoint inhibitors.

MYC, located on chromosome 8q24.21, is a regulator gene that regulates the expression of approximately 15% of human genes by recruiting histone acetyltransferases and binding enhancer box sequences (E-boxes) [6,7]. Malfunction of MYC, including *MYC* translocation involved in the development of Burkitt lymphoma, is found in cervix, colon, breast, stomach, and lung cancer[8–10]. MYC overexpression is observed in 41% of non-small cell lung cancer (NSCLC) and associated with loss of cell differentiation [9]. In addition to its classic function, MYC seems be involved in preventing immune cells from attacking tumor cells by inducing PD-L1 and CD47 expression [11]. Using cancer cells and mouse models, Casey et al. showed that (1) suppression of MYC in mouse and human tumor cells causes a reduction in the levels of PD-L1 mRNA and protein (2) MYC directly binds to the promoters of the PD-L1 genes (3) MYC inactivation in mouse tumors down-regulates PDL1 expression and enhances the anti-tumor immune response [11]. This finding suggests that when combined with PD-L1 expression, MYC and PD-L1 double-positive lung cancer patients may exhibit unique clinical characteristics. MYC could be a surrogate biomarker for response prediction for therapy with immune checkpoint inhibitors.

To explore this possibility, we investigated the correlation of MYC and PD-L1 expression using human lung cancer tissue and evaluated the clinical implications of MYC and PD-L1 double positivity in lung cancer tissues.

2. Materials and methods

2.1 Cell lines, plasmids, and antibodies. Lung cancer cells were obtained from ATCC (Manassas, VA, USA) or the Korean Cell Line Bank (Seoul, Korea). pcDNA3-cMYC was a gift from Wafik El-Deiry (Addgene plasmid #16011) and pcDNA3 was the negative control [12]. Anti-PD-L1 (22C3) was obtained from DAKO and anti-cMYC antibody (Y69) was from Abcam® (ab32072). Unless otherwise stated, antibodies were from Cell Signaling Technology (Danvers, MA, USA).

2.2 Study population. The study used 84 lung cancer tissues that met following criteria: (1) histologically confirmed as lung cancer, (2) from patients who underwent curative surgical resection from 2005 to 2010; and (3) from patients who agreed to provide and use of tissues to the institutional tissue archive. This study was approved by the institutional review board (IRB) of Severance Hospital (IRB No. 4-2016-1077) and was carried out in compliance with the Declaration of Helsinki and Korean GCP guidelines.

2. *Immunoblotting and immunohistochemistry(IHC)*

Immunoblotting was as described elsewhere [13]. Expression of PD-L1 in lung cancer tissues was determined using the DAKO FDA-approved PD-L1, 22C3 PharmDx™ protocol using the Dako Automated Link 48 platform. PD-L1 expression was considered positive if ≥ 50% of viable tumor cells exhibited membrane staining with any intensity. Expression of MYC in samples was analyzed by IHC using the LABS®2 System (Dako, Carpinteria, CA, USA) according to the manufacturer's instructions using primary antibodies against MYC (Y69) (ab320720). Scoring for MYC expression was as in Volm et al. [14].

2. *Statistical analysis*

Clinically significant differences in tested protein expression levels