Biochemical and Biophysical Research Communications xxx (2018) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Frequencies and expression levels of programmed death ligand 1 (PD-L1) in circulating tumor RNA (ctRNA) in various cancer types

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ARTICLE INFO

Article history:
Received 5 April 2018
Accepted 14 April 2018
Available online xxx

Keywords:
PD-L1
Circulating tumor DNA
Liquid biopsy
Cancer

ABSTRACT

Background: Precision medicine and prediction of therapeutic response requires monitoring potential biomarkers before and after treatment. Liquid biopsies provide noninvasive prognostic markers such as circulating tumor DNA and RNA. Circulating tumor RNA (ctRNA) in blood is also used to identify mutations in genes of interest, but additionally, provides information about relative expression levels of important genes. In this study, we analyzed PD-L1 expression in ctRNA isolated from various cancer types. Tumors inhibit antitumor response by modulating the immune checkpoint proteins programmed death ligand 1 (PD-L1) and its cognate receptor PD1. The expression of these genes has been implicated in evasion of immune response and resistance to targeted therapies.

Methods: Blood samples were collected from gastric (GC), colorectal (CRC), lung (NSCLC), breast (BC), prostate cancer (PC) patients, and a healthy control group. ctRNA was purified from fractionated plasma, and following reverse transcription, levels of PD-L1 expression were analyzed using qPCR.

Results: PD-L1 expression was detected in the plasma ctRNA of all cancer types at varying frequencies but no PD-L1 mRNA was detected in cancer-free individuals. The frequencies of PD-L1 expression were significantly different among the various cancer types but the median relative PD-L1 expression values were not significantly different. In 12 cases where plasma and tumor tissue were available from the same patients, there was a high degree of concordance between expression of PD-L1 protein in tumor tissues and PD-L1 gene expression in plasma, and both methods were equally predictive of response to nivolumab.

Conclusions: PD-L1 mRNA can be detected and quantitated in ctRNA of cancer patients. These results pave the way for further studies aimed at determining whether monitoring the levels of PD-L1 mRNA in blood can identify patients who are most likely to benefit from the conventional treatment.

1. Background

Past efforts in improving cancer treatment have largely

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https://doi.org/10.1016/j.bbrc.2018.04.120
0006-291X/

Abbreviations

QOL	quality of life
CTC	circulating cancer cells
CtDNA	circulating tumor DNA
CtRNA	circulating tumor RNA
PD-L1	proteins like programmed death ligand 1
PD-1	programmed cell death 1
MAPK	mitogen-activated protein kinase
PI3K	phospho-inositide 3-kinase
HER2	human epidermal growth factor receptor 2
GC	gastric cancer
CRC	colorectal cancer
NSCLC	non−small cell lung cancer
BC	breast cancer
PC	prostate cancer
QPCR	quantitative polymerase chain reaction
DCT	delta Ct
ANOVA	analysis of variance

consisted of screening, development of new anti-cancer agents, multi-drug combinations and advances in the radiation therapy. A more recent approach is precision medicine, which takes individual variability into account in order to design personalized treatment strategies [1]. An important goal of precision medicine is to identify molecular markers indicative of therapy selection by analyzing the factors involved in the therapeutic effects and prognosis [2,3]. So far, such information has been obtained by analysis of genes and proteins in cancer tissue biopsies. However, the use of tissue biopsies has many problems, including possible sampling bias and a limited ability to monitor tumor markers in patients during the course of the therapy. In 1977, Leon et al. discovered that serum ctDNA levels were higher in patients with cancer [4], suggesting that the extra serum DNA in cancer patients originates from the tumor. Subsequent work confirmed this and established that the ctDNA reveals the same information about the patient's genes as that found in the tumor without an invasive tissue biopsy [5–7]. The genetic information from liquid biopsies resides in circulating cancer cells (CTC), circulating tumor DNA (ctDNA), circulating tumor RNA (ctRNA) and exosomes [8]. Cell-free or extracellular RNAs have been detected in many human bodily fluids, including serum/plasma, saliva, cerebrospinal fluid, synovial fluid, tear fluid, amniotic fluid and urine [9]. For those genes that are expressed, ctRNA contains the same mutational information as does ctDNA, but in addition, can also provide information about the quantitative expression levels of genes of interest.

It is now well established that tumors can inhibit autoimmune antitumor activity by modulating the expression of inhibitory factors and immune checkpoint proteins like programmed death ligand 1 (PD-L1) and its cognate receptor, programmed cell death 1 (PD-1) [10–14]. As an immune suppression mechanism, the expression of PD-L1 is elevated in many types of cancer and is often correlated with poor patient prognosis and predictive of responses to the antibodies against PD-1/PD-L1 [15–17]. Therapies that block this interaction have demonstrated very promising clinical activity in several cancer types [11,12,17,18]. Excluding currently used antibodies for such therapies, inhibition of PD-L1 expression could also have a therapeutic effect. As PD-L1 expression is regulated by mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/AKT signaling pathways, inhibition of these pathways should reduce PD-L1. Indeed, receptor tyrosine kinase inhibitors result in better treatment outcome in lung cancers with high-expression of PD-L1 [17,19,20]. The PD-1 inhibitor, pembrolizumab is now under evaluation in 13 trials across more than 30 types of cancer including bladder, colorectal, gastric, head and neck, melanoma, non-small and small cell lung (NSCLC), renal, pancreatic, prostate, triple negative and estrogen-receptor positive human epidermal growth factor receptor 2 (HER2)-negative breast, gynecologic, and hematologic malignancies [21].

There has to date been no report about using ctRNA to detect PD-L1 expression and there are only a few studies across the various cancers about ctDNA [22]. The purpose of this study was to analyze the frequency and level of PD-L1 expression in ctRNA isolated from various cancer types.

2. Methods

2.1. Patient samples and samples transportation

A total of 760 patient samples were blinded and accessioned by Liquid Genomics, Inc.(later acquired by NantHealth, Inc.) The cancer types comprising the samples included; 44 gastric cancers (GC), 212 colorectal cancers (CRC), 320 non-small cell lung cancers (NSCLC), 24 breast cancers (BC), and 88 prostate cancers (PC). Only cancer types (not histologic subtypes) were known of the samples. All cancer patients had distant metastases and had scheduled chemotherapy. Patient samples were from University of Southern California Norris Comprehensive Cancer Center, Memorial Cancer Institute or University Hospital Essen, West German Cancer Center. This study was approved by the Institutional Review Board of each facility. Ten milliliters of blood were collected in each of two tubes containing a proprietary nucleic acid preservation cocktail and transferred to Liquid Genomics, Inc. as soon as possible. After blind accessioning, all samples proceeded to the isolation process within five days after collection. A two-dimensional bar code was placed on all plasma samples for automatic identification.

2.2. Fractionation of plasma and extraction of ctRNA

Whole blood in 10 mL tubes was centrifuged to fractionate plasma at 16,000 rcf for 20 min ctRNA was extracted from 2 mL of plasma with a proprietary in-house developed protocol especially designed to remove potential contaminating blood cells during the extraction. All nucleic acids were kept in bar-coded matrix storage tubes. RNA was stored at −80 °C or reverse-transfected to complementary DNA (cDNA) and cDNA was stored at −4 °C.

2.3. The detection of PD-L1 with quantitative real-time PCR

Quantitative real-time PCR was used to detect the expression of PD-L1 in ctRNA. We designed the primers to investigate the expression of PD-L1. Amplification was performed in 10 μL reactions containing 2 μL cDNA, the primers, the probe, and the reaction mix with qPCR and an in-house developed assay. β-actin was used as an internal control. PD-L1 delta-Cts (dCts) were calculated from the PD-L1 Ct value by using β-actin as the positive control dCts were the Ct value of PD-L1 subtracted by the Ct value of β-actin. Next we calculated K using universal human reference (UHR) as a positive control. With using this K, we can reach to the Relative PD-L1 Gene Expression.

Relative PD-L1 Gene Expression= (2^-dCT)*K

Relative PD-L1 Gene Expressions were compared across various cancers.

Please cite this article in press as: T. Ishiba, et al., Frequencies and expression levels of programmed death ligand 1 (PD-L1) in circulating tumor RNA (ctRNA) in various cancer types, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.120