Liquid biopsy in targeting gene polymorphism related to the response within immunocheckpoint inhibitors therapeutic regimen

Introduction

Liquid biopsy (LB) is the most superior diagnostic method for the determination of the tumour signature in blood [1].

Breast cancer is the most common female cancer worldwide. Only in 2018, it reached the top rate among 25 cancers for 40 countries in Europe (523,000 cases, within 3.91 million new cases of cancer per year in both genders) [2]. In simplified words, the prognosis depends on the tumour type (lumina, basal-like, triple-negative) and its stage (histological grade, axillary lymph node involvement and distant metastasis) at the time of diagnosis. Despite low immunogenicity in breast cancer [3], there is a rising interest in anti-programmed dead receptor 1 and programmed dead ligand 1 (anti-PD1/anti-PD-L1) treatment in a special subset of triple-negative breast cancer (TNBC) and human epidermal growth factor receptor 2 positive (HER-2 positive) [4–6]. In March 2019, FDA approved Atezolizumab (Tecentriq) for adult patients with unresectable, locally advanced or metastatic triple-negative breast cancer (TNBC) the tumours of which express PD-L1 as determined by an FDA-approved test [7].

PD-1 firstly identified by Ishida in 1992 [8] is a transmembrane glycoprotein located in tumour cells, cytotoxic T-lymphocytes, natural killer (NK), B-lymphocytes, monocytes and other tissues infiltrating lymphocytes (TILs) [9]. PD-1 receptors linked with their ligands PD-L1 identified in 1999 [10] on an antigen presenting cell (APC) inhibit antitumour activity. PD-1/PD-L1 axis block activation of cytotoxic T-cell. This blockade plays a critical role in tumour resistance mechanisms. The suspension of PD-1/PD-L1 axis can restore T-cell and promote immunity against tumour. Antitumour effect exerted via immunocheckpoint inhibitors (Atezolizumab,
Avelumab, Nivolumab, Pembrolizumab) is based on the promotion of a cell-mediated immune response. To date, for the purpose of pretreatment qualification, FDA-approved only the PD-L1 expression level, as determined by an IHC based on the VENTANA assay [7] for a primary or metastatic tissue source. It is still unclear if PD-1(PDCD1)/PD-L1(Pdcd1l2) gene single nucleotide polymorphism (SNP) could be a predictor for immunotherapy inhibition treatment, especially in the context of breast cancer risk and prognosis [11, 12]. The frequencies of rs36084323 GG genotype and rs2227981 CT genotype can affect the susceptibility of breast cancer [12]. The human gene encoding PD-1 is located on 2q37.3 [12].

Programmed death-1 (costimulatory molecule, PD-1, CD279) gene polymorphism/ PDCD1 (coding for programmed cell death-1)

Loci of PD-1 gene might be a potential biomarker for predicting susceptibility to therapeutic markers for cancer treatment. However, the identification of biomarkers able to predict a clinical benefit of PD-1/PD-L1 inhibitors seems to be a challenge. To apply liquid biopsy into minimal invasive stratification, single nucleotide polymorphism (SNP) should be designated. Seven susceptibility loci for immunotherapy effect in breast cancer patients can be considered: rs41386349, PDCD1 (rs7421861), PD-1.1 (rs36084323), PDCD-1.3 (rs11568821), PDCD-1.5 (rs2227981), PDCD-1.6 (rs10204525), PDCD-1.9 (rs2227982). Previous studies have discussed methods and combined biomarkers from different points of view: tumour mutation and neoantigens burden as well as some oncogene mutations like EGFR, ALK, KRAS and STK11 [13].

However, PD-L1 expression measured using the IHC is the only FDA-approved test. Lower PD-1 expression has been found to be associated with G of rs10204525 [14].

Certain single nucleotide polymorphisms (SNPs) in genes such as PDCD1 (coding for programmed cell death)

Rs41386349, this SNP was previously considered as a hot point for Grave’s disease and Addison’s disease [15], rheumatoid arthritis (RA) [16], Kawasaki disease [17], multiple myeloma [18] and variant A for rs41386349 affects susceptibility of chronic HBV [19]. Whereas, only PD-L1 rs1411262C/T gene polymorphism in the case of Grave’s disease and Addison’s disease [15], increased rheumatoid factor seropositivity [16] and a higher probability of Kawasaki disease [17] was discovered. The multiple myeloma occurs more often with a PDCD1 GCC/GCC haplotype (rs36084323/rs41386349/rs2227982) variant [18].

PDCD1 (rs7421861) has been studied in case of esophageal cancer [20] and chronic HBV infection, its oncogenicity [19] as well as RA [16]. PDCD1 gene polymorphisms influence the severity of a disease and are associated with distant metastasis, higher TNM stage, higher PD-1 gene and plasma levels in esophageal cancer patients and risk of esophageal cancer in general [20]. This gene variability may play a role in hepatocarcinogenesis caused by chronic HBV infection [19]. Tseng CC et al. have found higher expression of PDCD1 in RA compared to controls. However, PDCD1 gene polymorphisms were similar in this study [16].

PD-1.1 (rs36084323) has been considered in a case of breast cancer [21], epithelial ovarian cancer [22] esophageal cancer [20], NSCLC [23], overall cancer risk [24], RA [16] pregnancy losses [25] and aplastic anaemia [26]. Therefore, significantly lower frequencies of PD-1.1 GG have been presented in women with breast cancer [21]. Rs36084323 polymorphism predicts epithelial ovarian cancer development [22]. No association with esophageal cancer metastasis [20], no differences in the distribution of the PD-1.1 alleles in NSCLC [23], no association between overall cancer risk have been obtained [24]. For autoimmune diseases, such as RA, rs36084323 decreased an inadequate response to conventional synthetic disease-modifying antirheumatic drugs [16]. Hayashi Y. et al. have evaluated the association of genetic variants of PD1 with recurrent pregnancy loss with significantly higher frequencies of rs36084323 in women with two or more pregnancy losses [25]. Polymorphism of PD-1.1 has been rare in the case of patients with aplastic anaemia [26].

PDCD-1.3 (rs11568821) gene polymorphism has been considered to be related to an overall cancer risk [24, 27] and SLE [28]. Researchers have found a decreased overall cancer risk in case of PDCD-1.3, variant TC [24, 27]. PD1.3GG genotype and G allele have been significantly more frequent in SLE patients [28]. Prokunina et al. have found that this genetic variant would affect PD-1 mRNA level by changing the binding affinity of RUNX (a transcriptional factor of PD-1) [29].

PDCD-1.5 (rs2227981) variant TT has been associated with a decreased risk of cancer [24, 27]. CT genotype has been significantly lower in breast cancer women [21], whereas an increase in the risk of cervical [30], T-allele of rs2227981 gene polymorphism reduced risk of epithelial ovarian cancer [22], lung [31], gastric [32], colon [33], thyroid cancers [34] and CC variant was significantly more frequent in patients suffering from SLE [28].
PDCD-1.6 (rs10204525) has been explored with respect to the associations between PD-1.6 gene polymorphisms and esophageal cancer [20, 35], overall cancer risk [24], aplastic anemia [26], juvenile idiopathic arthritis [36] and HBV infection [37]. GG genotype of rs10204525 polymorphism has increased the risk of esophageal cancer in contrast to the more common AA genotype which was associated with distant metastasis and higher PD-1 gene and plasma levels [20, 35]. Even in some other work, there is no relation between this SNP and overall cancer risk [24]. Furthermore, some variants of rs10204525 have been linked to aplastic anaemia [26], while the CT variant was associated with juvenile idiopathic arthritis and linked to Anti-CCP antibodies, RF, and the CHAQ score [36]. Next, subjects carrying minor allele G had a significantly decreased risk of getting infected with HBV and were associated with lower PD-1 expression [14], variant AA of PD-1 rs10204525 was prone to higher PD-1 expressions in tumour tissues, peri-tumour tissues and cirrhotic tissues [37]. GG genotype variant altered tumour necrosis factor-α (TNF-α) to increase levels in HBV patients [38]. PDCD-1.9 (rs2227982) has been evaluated in terms of breast cancer [39], ovarian cancer [40], ankylosing spondylitis [41], multiple myeloma [18] and chronic HBV infection and its oncogenicity[19]. This polymorphism increases the probability and severity of the disease in case of ankylosing spondylitis and breast cancer [39, 41]. Simultaneously, the same researchers have found that C > T variant reduces the risk of breast cancer [39] and raises the risk of ovarian cancer [40]. CC genotype variant was significantly correlated with a higher frequency of osteolysis [18]. This gene polymorphism may have a significant influence on hepatocarcinogenesis [19]. However, CC of rs2227982 variant had a shielding role in HBV infection [14].

**Conclusions**

In summary, there are no reports on PD-1 gene polymorphism and its association with immunotherapy favourable outcome susceptibility. Seven susceptibility loci was considered: rs41386349, rs7421861, rs36084323, rs11568821, rs2227981, rs10204525, rs2227982. The important question for developing next-generation anti-PD-1/PD-L1 antibodies is whether the therapeutic effect can be predicted with SNP analysis using liquid biopsy. Nevertheless, three SNP may be taken into account in the breast cancer patient: rs36084323, rs2227981, rs2227982.

**Conflict of interest:** The authors have no conflicts of interest to declare regarding this study.

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