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Review

REVIEW

Role of tumor gene mutations in treatment response to immune checkpoint blockades

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Abstract

Early studies shed light on the immune suppression of immune checkpoint molecules in the cancer microenvironment, with later studies applying immune checkpoint blockade (ICB) in treatment of various malignancies. Despite the encouraging efficacy of ICBs in a substantial subset of cancer patients, the treatment response varies. Gene mutations of both tumor cells and immune cells in the tumor microenvironment have recently been identified as potential predictors of the ICB response. Recent developments in gene expression profiling of tumors have allowed identification of a panel of mutated genes that may affect tumor cell response to ICB treatment. In this review, we discuss the association of the ICB response with gene expression and mutation profiles in tumor cells, which it is hoped will help to optimize the clinical application of ICBs in cancer patients.

Key words: gene mutation; immune checkpoint blockade; tumor

Introduction

With the emergence of immune checkpoint blockades, the field of anti-cancer therapy has currently shifted its focus to antagonizing agents that target inhibitory signaling molecules on tumor and immune cells.[1,2] Once the immune suppression of immune checkpoints is released by immune checkpoint blockades (ICBs), the immune system is then activated, which can be best characterized with the inflammatory response observed at tumor sites. In addition to the most intensively studied ICBs such as the antibodies targeting CTLA-4 (cytotoxic T lymphocyte-associated antigen-4),[3,4] PD-1 (programmed cell death-1),[5–7] and PD-L1 (PD-1 ligand),[8,9] a wide range of other ICBs have also exhibited striking clinical benefits in cancer patients. An increasing number of ICBs are currently under evaluation for treatment of various advanced diseases, many of which are approved by the Food and Drug Administration each year.[10–14]

Despite the considerable clinical benefit of ICBs in a substantial subset of cancer patients, their efficacy varies. It is of paramount importance to identify predictive biomarkers for ICB responses. Previous reports have suggested the accumulation of gene mutations as a tumor hallmark. Although the mutational burden varies

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widely both within and among cancer types,[15] a subset of cancer patients has demonstrated similar gene mutation profiles. It is therefore conceivable that gene alterations play a functional role in tumor response of ICBs.

Application of genomic and transcriptomic analyses can facilitate accessible gene expression profiling of tumors to identify potential prognosis predictors.[16–19] Tumor-related genes described thus far are mostly involved in cytokine and chemokine signaling pathways. Many of these genes including EREG and AREG, have been reported to predict response to chemotherapy in cancer patients[20,21] and their differential expression may also affect the treatment response to ICBs.[22,23] Compared with the traditional quantitative immunohistochemical (IHC) method, the identification of gene expression can establish a more comprehensive response profile, in that it simultaneously evaluates substantial parameters including expression of immune checkpoint molecules and inflammatory genes. However, the gene expression assay fails to present spatial and structural details that may also serve as prognostic factors.[24] The integration of multiple detecting platforms is therefore warranted to provide comprehensive data to predict ICB response.[24]

In this review, we briefly discuss the association of ICB response with gene expression and mutation profiles in tumor cells. With a growing body of study on the underlying mechanism of the association, we also discuss the intratumoral mutational burdens and mutational heterogeneity, which aids the stratification of ICB treatment to minimize the potential treatment resistance.

Response to ICB in the context of tumor cell gene expression

Intense investigation is currently under way into potential predictive biomarkers of the response to ICBs. Akiyama *et al.* established a gene panel consisting of 164 immune therapy response-associated genes from 1000 tumors based on whole-exome sequencing and gene expression profiling.[25] Multiple molecular predictors have been proposed of tumor response to the ICB. High levels of PD-L1 expression, IFN-γ induced gene expression in tumor cells, and CD8+ T cell infiltrates have all been detected in patients responsive to immune checkpoint therapy.[26,27] Some studies of tumor biopsies have suggested a predictive role for CD8+, CD4+, PD-1+, and PD-L1+ in tumor cells in terms of the therapeutic response to ICBs.[28,29]

Tumor expression of PD-L1

Based on the PD-1/PD-L1 signaling pathway, it is conceivable that anti-PD-1/anti-PD-L1 therapy is largely associated with expression of PD-L1 in tumor cells or tumor-infiltrating immune cells. Previous studies have demonstrated a correlation between tumor expression of PD-L1 and response to PD-1 inhibitors in some cancer types.[5,6,27,30,31] Expression of PD-L1 on tumor cells varies from absent in Merkel cell carcinomas to 100% in liposarcomas and chondrosarcomas.[32] Meanwhile, triple-negative breast cancers and colon cancers with high microsatellite instability (MSI) have significantly higher PD-L1 expression than that seen in non-triple-negative breast cancers and microsatellite stable tumors, respectively.[32] In a clinical trial evaluating patients treated with the anti-PD-1 agent pembrolizumab, the higher expression of PD-L1 on tumor cells was associated with a better outcome compared with tumors with low PD-L1 expression.[33] In this trial, a proportion score of ≥50% was used as the cut-off value, referred to as the PD-L1 expression on at least 50% of the tumor cells. A response rate of around 90% to nivolumab has been observed in patients with Hodgkin's lymphoma with amplified PD-L1/PD-L2.[11] Moreover, higher levels of CTLA-4 mRNA are also found to indicate better response to both anti-CTLA-4 and anti-PD-L1 therapy.[22,27] The PD-L1 copy number gains in non-small-cell lung cancer (NSCLC) cells display higher expression of PD-L1, which can potentially serve as a predictor of response to anti-PD-1/PD-L1 therapy. However, PD-L2 copy number gains fail to result in augmented PD-L2 expression.[34] Kim *et al.* later identified nine genes related to immune checkpoints, including PD-1, PD-L1, and CTLA-4, the expression of which is positively correlated with the number of infiltrating lymphocytes in metastatic breast cancers, suggesting a potential predictive function of these gene markers.[35] Taken together, this evidence suggests that expression of PD-L1/PD-L2 and CTLA-4 can be used as a target for gene expression-based immunotherapy.

However, in some reports, tumors classified as PD-L1-positive did not respond and the baseline PD-L1 status was not an independent predictor of treatment response.[14,27,36,37] Such differing reports can probably be attributed to use of different tumor types and histologic categories, and heterogeneity of PD-L1 expression within the tumors themselves. Differences may also reflect the limitations of IHC staining as the detection method used for PD-L1 expression, given that IHC staining can be performed on different staining platforms with different antibodies. One such example is the comparison staining performed by Smith *et al.*[38] They compared the performance of two PD-L1 clones (SP263 and E1L3N) and found that SP263 IHC assays had higher sensitivity and a wider dynamic range than E1L3N assays. On the other hand, Carbognin *et al.* found that the cut-off value for PD-L1 positive melanomas could have an impact on study conclusions.[39]

Despite the dramatic efficacy of PD-1/PD-L1 blockades, some patients fail to respond to the initial PD-1/PD-L1 therapy, which is known as primary resistance.[7,40] Moreover, a subset of responders eventually develop acquired resistance after initial treatment. Therefore, it is of paramount importance to identify biomarkers related to ICB resistance. PD-1/PD-L1 blockade responses can be greatly impacted by biomarkers such

as tumor neoantigens and MSI, which we briefly describe in the following discussion. The tumor PD-L1 expression serves as another biomarker to predict responses to PD-1/PD-L1 blockade, the clinical application of which needs in-depth consideration for tumor types.[41] The tumor PD-L1 expression has been identified as an acquired resistance mechanism for tumor cells in response to immunotherapies.[1,42] Importantly, it has to be addressed that one single biomarker is insufficient to predict drug resistance and combining different biomarkers is therefore a valuable option for prediction.

Tumor expression of MHC

Given that T cells recognize tumor cells through recognition of peptides that bind to the major histocompatibility complex (MHC), theoretically, decreasing expression of MHC I would promote tumor survival and reduce ICB efficacy. An early study by Restifo *et al.* found that melanoma patients with β2 microglobulin deficiency, which led to the incompleteness of MHC I, did not respond to T-cell-based immunotherapy.[43] A growing body of literature has reinforced this finding that the loss of MHC I expression on tumor-infiltrating immune cells leads to the immune evasion of melanoma cells and their acquired resistance to anti-PD-1 agents following the initial response.[44–46] On the other hand, MHC II expression on melanoma cells has also been found to predict response to anti-PD-1 treatment,[47] and expression of MHC II on some melanoma cell lines can be restored with IFN-γ treatment.[48]

Response to ICB in the context of tumor cell gene alterations

Multiple oncogenic events have been reported to contribute to acquisition of the phenotype and malignant properties of cancer. Developments in techniques for identification of genetic abnormalities, have allowed identification of a subset of cancer patients with similar gene mutation profiles. It is therefore conceivable that gene alterations play a functional role in tumor response to ICBs. Although no clear associations have been established, this review focuses on the potential gene mutations that are predictive of therapeutic sensitivity to ICBs.

Ras and RAF gene mutation

The Ras/Raf/MEK/MAPK pathway is involved in proliferation of both normal and tumor cells, and is therefore associated with tumorigenesis and tumor progression.[49] The RAS oncogene family includes KRAS, NRAS, and HRAS, which have all been characterized as potential predictors of anti-epidermal growth factor receptor (EGFR) treatment response in colorectal cancer.[50] KRAS mutations occur in approximately 20% of NSCLC,[51] and are associated with poor prognosis in cancer patients.[52,53] Patients with KRAS mutations exhibit intrinsic resistance to anti-EGFR treatment,[54] but are sensitive to MEK/ERK inhibitors such as selumetinib.[55,56] Previous studies have shown that patients with KRAS mutations, especially those with concomitant TP53 mutations, had increased PD-L1 expression and remarkable clinical response to PD-1 inhibitors.[57] Compared with patients with wild-type TP53, there was a significantly higher proportion of patients with mutations in TP53 who exhibited high PD-1 expression (10.9% compared with 34.2%, $P = 0.023$). As for NRAS mutations, patients with NRAS mutant melanoma displayed a significantly worse overall survival of 8.2 months compared with patients with wild-type BRAF/NRAS (15.1 months, $P = 0.004$).[58] And it has been reported that patients with NRAS mutation-positive melanomas demonstrate better disease control (median progression-free survival 4.1 vs. 2.9 months, $P = 0.09$) when treated with CTLA-4 and PD-1 antibodies,[59] and prolonged overall survival (OS, 12.1 vs. 8.03 months) when treated with ipilimumab.[60] Kirchberger *et al.* suggested that immune checkpoint inhibition induced encouraging responses in both NRAS-mutated and NRAS wild-type melanoma patients; however, worse OS was observed in the case of NRAS mutation, but this could be overcome by additional use of MEK inhibitors.[61]

The predictive value of BRAF mutation in the context of treatment response to ICBs remains incompletely defined. BRAF mutation status appears to be irrelevant of disease progression or OS,[62,63] but some reports have identified BRAF mutation as a negative prognostic indicator.[62,64] Although immune checkpoint inhibitors have significant benefitted cancer patients in randomized clinical trials,[3,4,14,65,66] the BRAF mutation status is independent of the response difference.[67,68] A recent report, however, suggested that the BRAF mutation is moderately related to worse survival in melanoma patients treated with ipilimumab.[69]

EGFR gene mutation

EGFR mutations are often seen in patients with lung cancer, and different mutation types can potentially affect the treatment response to anti-cancer therapies.[70–72] The T790M EGFR mutation has been reported to account for more than half of patients resistant to first-line EGFR tyrosine kinase inhibitors.[73] Moreover, T790M mutation status is also associated with PD-L1 expression levels. Previous studies have detected a lower rate of PD-L1 positivity in patients with EGFR mutations.[74] And in cancer patients with PD-L1 overexpression who are treated with pembrolizumab, those with EGFR mutations have exhibited significantly shorter OS than those with wild-type EGFR.[75] In patients with EGFR mutations, increased PD-L1 expression indicates higher response rates to immuno-oncology agents.[76,77] IHC results have suggested a high expression profile of PD-L1 and PD-1 in tumor specimens of patients with EGFR-mutant NSCLC, suggesting that PD-1/PD-L1

immunotherapy could potentially benefit such patients.[78] Lee *et al.* reported that compared with chemotherapy, immune checkpoint inhibitors failed to bring survival benefits in patients with EGFR-mutant NSCLC.[79] Moreover, anti-PD-1/PD-L1 therapy does not achieve a significant survival improvement in patients with EGFR mutations.[80] However, compared with ICB monotherapy, the concomitant use of PD-1 and CTLA-4 inhibitors can augment the immunogenicity of EGFR-mutant tumors and increase the treatment response.[81] The 2-year overall survival of advanced melanoma patients was 63.8% in a group of patients receiving nivolumab plus ipilimumab versus 53.6% in a group receiving ipilimumab monotherapy.[82]

Janus kinase (JAK) gene mutation

The JAK family includes four members, JAK1, JAK2, JAK3, and TYK2 (non-receptor protein-tyrosine kinase 2).[83] Multiple studies have explored the role of a gene locus adjacent to JAK2, the 9p24.1, the amplicon of which is often seen in both solid tumors and hematologic malignancies.[84,85] The emergence of JAK2 at the 9p24.1 gene site is crucial to PD-L1 expression, as JAK2 signaling has been found to increase PD-L1 expression.[85] A study has identified the synergistic amplification of PDL1/PD-L2/JAK2 as a potential predictor for the response to immune checkpoint therapy.[86] Furthermore, amplifications of the PD-L1 and JAK2 genes are mutually correlated, as observed after use of the JAK2 inhibitor TG-101348 which can cause a decrease in PD-L1 protein.[87] Both somatic and germline genomic alterations of JAK3 have been reported to promote PD-L1 activation in lung cancer cells and potentially impact their response to the PD-L1 immune checkpoint therapy.[88]

MSI

Resulting from an intrinsic deficiency in DNA mismatch repair (MMR), MSI often occurs throughout the genome in colorectal cancers.[89] Accumulation of DNA alterations is an essential step towards carcinogenesis, which reflects the somatic and germline variants involved in DNA damage and repair. Previous studies have identified use of MSI as a potential biomarker to predict sensitivity to anti-PD-1 therapy as a less than ideal treatment strategy in colorectal cancers.[90]

A phase II study suggested that the anti-PD-1 agent nivolumab induced encouraging anti-tumor activity in patients with MMR-deficient cancers.[91] A recent study presented a case of a cancer patient with high levels of MSI who demonstrated a striking response to another PD-1 inhibitor, pembrolizumab.[92] In this case, after the patient received the initial pembrolizumab treatment, her chest wall lesion was significantly softened with tumor tissue necrosis. There are also cases of other tumors, such as glioblastoma and extrahepatic cholangiocarcinoma, which lacked the expression of potential response predictors for PD-L1 inhibitors.[93,94] In a phase II clinical trial,[95] a more robust response to pembrolizumab was observed in patients with MMR-deficient colorectal cancer than in patients with proficient MMR, suggesting that cancers with intensive somatic mutations caused by MMR deficiency are susceptible to immune checkpoint blockade. A growing body of literature has reported similar results in an expanding tumor type profile,[95–99] including the left frontal glioblastoma with DNA-repair defects in which ICB treatment results in central nervous system (CNS) immune activation.[100]

Other gene mutations

Current knowledge on gene mutations as biomarkers predicting ICB response represents only the tip of the iceberg. Recent publications have highlighted the alterations of other potential predictive genes. A significant proportion of cancer patients with targetable mutations in SMO, DDR2, FGFR1, PTCH1, FGFR2, and MET have been reported to be eligible for certain checkpoint inhibitors.[101] Matsuo *et al.* identified a subset of patients with BRCA mutant epithelial ovarian, fallopian tubal, and primary peritoneal cancers who responded to nivolumab monotherapy.[102] Moreover, gene mutations of DNA polymerase epsilon (POLE) are also correlated with increased expression of certain immune checkpoint genes, indicating that cancers with POLE mutations are candidate predictors for immune checkpoint therapy.[103] More recently, Miao *et al.* found that loss-of-function mutations of the PBRM1 gene in clear cell renal cell carcinoma can lead to remarkable responsiveness to immune checkpoint therapy.[104] PTEN mutations and decreased expression of neoantigen genes have also been demonstrated as having potential predictive value for resistance to immune checkpoint therapy.[105] Ongoing studies are likely to result in development of an expanding repertoire of gene mutations.

Mechanism of the correlation between ICB response and gene mutations

Tumor neoantigens and mutational load

Neoantigens are also referred to as the proteins resulting from DNA mutations within tumor cells that can be recognized as foreign proteins by the immune system.[106–113] Previous reports have emphasized that tumors with higher mutational frequency generate larger numbers of neoantigens than tumors with lower mutational frequency.[114] When neoantigens are released from tumor cells, cytotoxic T lymphocytes engage the tumor microenvironment, exert killer functions on the tumor cells, and induce tumor cell apoptosis, which in turn promotes release of more neoantigens and enhances the anti-tumor activities of the immune system.[115] To reach maximum anti-tumor efficacy, the anti-tumor therapy needs to overcome the suppressive tumor microenvironment including the inhibitory immune checkpoint molecules.

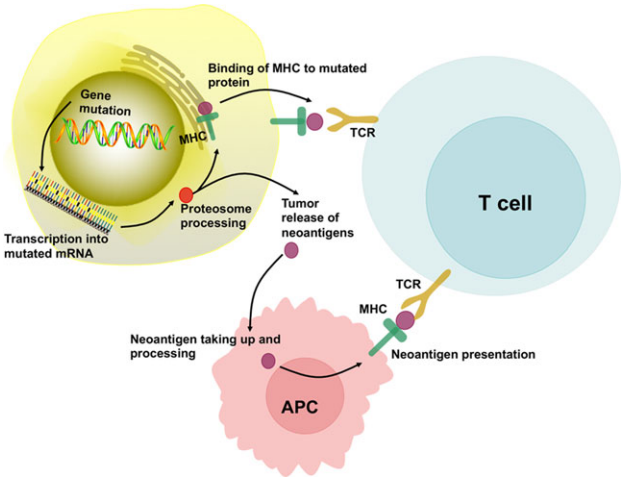

Figure 1. Simplified overview of the interaction between tumor neoantigens, T cells, and antigen-presenting cells (APCs). MHC, class I major histocompatibility complex; TCR, T cell receptor.

Figure 1 illustrates the interaction between tumor neoantigens, T cells, and antigen-presenting cells.

To understand the key to the success of ICBs, it is important yet challenging to identify antigens uniquely expressed on tumor cells and not on normal tissues. One approach is application of tumor mutational burden to represent mutated peptides on T cells that exist only on tumor cells and not the normal genome.[114] Correlations of durable responses to ICBs with mutational load and neoantigens have long been confirmed,[106,114] with some evidence suggesting that the mutational load may be more accurate than PD-L1 IHC in predicting the presence of clinical variables such as the number of tumor infiltrating lymphocytes.[106] Sequencing of tumor genomes can be used to predict the clinical benefit of ICBs because cancer patients with higher mutational loads have demonstrated improved OS after receiving ICBs.[116]

Previous studies on cancer-related gene profiles have demonstrated a significantly higher tumor mutation burden in immunotherapy responders compared with non-responders.[9,117] On the other hand, Hellmann *et al.* used whole-exome sequencing to evaluate the therapeutic efficacy of PD-1 plus CTLA-4 blockade in NSCLC patients, and found that patients with higher tumor mutation had improved objective response and progression-free survival.[118] It has been reported that melanoma cells are prone to damage by ultraviolet radiation, which causes an accumulating number of mutations in the melanocyte genome, leading to a higher level of mutational load.[119–121] In this subset of melanoma patients, higher mutational load is associated with better response to anti-CTLA4 therapy.[22] However, in one subtype of melanoma, the desmoplastic melanoma, no significant difference was detected between mutational loads in PD-1 blockade responders and non-responders.[122]

Although a higher mutational load is reported to be correlated with a durable clinical response to CTLA-4 blockade, tumor mutational load alone is not sufficient to indicate the clinical benefit, because not all tumors with high mutational loads respond to immune checkpoint therapy.[117] Furthermore, the mutation burden varies dramatically within tumors and among different tumor types, reflecting vast differences in the DNA-damage-repairing abilities of different tumors.[15]

Intratumoral mutational heterogeneity

In addition to high levels of neoantigens, therapeutic sensitivity to ICBs is also associated with low intratumoral heterogeneity.[113] Tumors with DNA damage response-deficiency exhibit random gene mutations, leading to profoundly high intratumoral heterogeneity.[123] High levels of mutational heterogeneity potentially enhance the interaction between tumor antigens and MHC molecules, and consequently induce the antigen presentation to T cells. Moreover, heterogeneous gene mutations increase the chance of tumor cells being identified by T cells by widening the T-cell killing repertoire, including recognition of hidden neoantigens by ICBs.[124] Safonov *et al.* recently suggested that the mutational heterogeneity is negatively associated with some immune metagenes in breast cancers, and that mutational heterogeneity leads to minimal immune cell infiltration, pointing to an escape mechanism of tumor cell from immune surveillance.[125] Therefore, more immunotherapy strategies are warranted to activate the anti-cancer immune activities against a genetically diverse neoplastic population.

Perspectives

An accumulation of new literature in recent years has reported therapeutic resistance to ICBs in cancer patients, which is one of the main obstacles in application of ICBs. Several mechanisms for such resistance have been proposed including PTEN loss which reduces the intratumoral infiltration and tumor killing of T cells, leading to resistance to anti-PD-1 or anti-CTLA-4 treatment in this subset of patients.[126] Immune resistance has also been detected in melanoma patients with the loss of β2-microglobulin.[43] Using whole genome shotgun (WGS) technology, patients failing to respond to anti-PD-1 therapy display increased expression of genes involved in the carcinogenesis process such as epithelial mesenchymal transition.[127] All these efforts provide new insights into the mechanism of drug resistance, suggesting that the gene mutation may be an attractive biomarker for predicting therapeutic efficacy.

Another challenge is how we broaden the utility of gene mutations in immune checkpoint therapy. One avenue is to identify more potential mutated genes that would have an impact on treatment response. One such strategy, genomic analysis, has been refined in recent decades and introduces new opportunities for cancer treatment. Genomic analysis technology, such as next-generation sequencing, can help to identify expressed

mutations of tumors and predicts candidate peptides that may still remain after ICB treatment.[128] Another potential option is improvement of the cancer vaccine concept. Cancer vaccines involve injection of cancer-specific elements into patients to induce anti-tumor immune responses. To achieve an adequate and durable T cell response, multiple vaccinations and incorporation of MHC class II peptides are required.[129,130] Study on these subjects will undoubtedly provide new perspectives for improvement of the ICB treatment response.

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Conflict of interest statement

None declared.

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