Neoantigens in Cancer

Subjects: Oncology

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The clinical benefits of immune checkpoint blockage (ICB) therapy have been widely reported. In patients with cancer, researchers have demonstrated the clinical potential of antitumor cytotoxic T cells that can be reinvigorated or enhanced by ICB. Compared to self-antigens, neoantigens derived from tumor somatic mutations are believed to be ideal immune targets in tumors. Candidate tumor neoantigens can be identified through immunogenomic or immunopeptidomic approaches. Identification of neoantigens has revealed several points of the clinical relevance. Hence, immunotherapies using vaccines or adoptive T-cell transfer targeting neoantigens are potential innovative strategies. However, significant efforts are required to identify the optimal epitopes.

Keywords: neoantigens ; ICB ; vaccines

1. Introduction

Clinical Significance of Tumor Neoantigens

Tumor-specific somatic mutation-derived antigens (neoantigens) are newly synthesized in tumors and recognized as non-self. By targeting neoantigens, the T cells can attack and kill tumors [1][2][3]. Clinical studies have reported successful therapeutic outcomes of immune checkpoint blockage (ICB) for tumor treatment [4][5]. Various immune cells show anti-tumor immune responses in the tumor microenvironment and lymph nodes, immune cells with direct tumor killing activity are essential for the eradication and suppression of proliferating tumor cells. In particular, $CD8^+$ T lymphocytes exhibit tumor selectivity and high cytotoxic activity. $CD8^+$ T cells become dysfunctional following chronic antigen exposure, and ICB treatment reinvigorates tumor-specific T cells by inhibiting signaling-mediated suppression. $CD8^+$ T cells often recognize over-expressed self-antigens in tumors, such as cancer testis antigens, exogenous onco-virus antigens, and tumor neoantigens [6]. Since $CD8^+$ T cells are educated to have central tolerance, viral-associated antigens or neoantigens are expected to be the ideal targets.

A large number of tumor DNA mutations potentially yield mutated peptide sequences. Tumor mutation burden (TMB) alters neoantigen load and immunogenicity; hence, tumors exposed to mutagens, such as skin and lung tumors (UV and cigarette, respectively), are proactively treated by ICB [7][8][9]. Additionally, ICB therapy reinvigorates neoantigen-specific T-cells, supporting their importance in killing tumor cells [10]. Indeed, a higher TMB is associated with clinical responses to ICB [8][11]. Mismatch repair-deficient tumors, which likely accumulate mutations during cell division, are susceptible to ICB treatment [12][13]. Tumor inhibition by ICB is partly accounted for by indel mutations and missense mutations. Therefore, microsatellite instability (MSI) may be a plausible biomarker for ICB therapy [14]. In contrast, patients respond variably to ICB treatment, regardless of the mutation burden. Since the responses to neoantigens vary in patients, the tumor type and mutation burden may not be the only factors influencing these responses [15]. In fact, a high mutation burden is reportedly a risk factor for multiple myeloma [16]. Given the tumor diversity, anti-tumor immune responses mediated by neoantigens need to be thoroughly investigated.

2. Neoantigen-Specific T Cell Responses

2.1. Reactivity of Neoantigen-Specific T Cells after Vaccines

After neoantigen candidates are identified by in silico prediction or mass spectrometry (MS) analysis, the next important step is to determine if the epitope can be directly recognized by T cells. Generally, in mouse experiments, synthesized peptides or coding RNAs are used to immunize and T cell reactivity against cognate peptides is monitored by IFN-γ production [17][18][19][20][21]. The tumoricidal potential against widely examined tumor cell lines can be evaluated regardless of the adjuvants used. Moreover, even though these epitopes are predicted to have high MHC-I affinity, synthetic long peptides or long neoepitope-coding RNA vaccines can elicit MHC-II-restricted $CD4^+$ T cell responses. With regard to the role of these neo-Ag specific $CD4^+$ T cells in anti-tumor immunity, it has not been explained completely. Since most tumors

lack MHC-II, tumor-infiltrating antigen-presenting cells (APCs) should express tumor-derived antigen including neo Ags on MHC-II. Therefore, $CD4^+$ T cells may help $CD8^+$ CTL via APC activation by CD40 ligand as well as IL-2 and IL-21 secretion [22][23][24]. Furthermore, several reports support the direct tumoricidal activity of $CD4^+$ T cells against certain MHC-II expressing tumors including neoantigens [25][26][27]. In fact, mutant MHC-II neoepitope vaccine elicited anti-tumor response in $CD4^+$ T cell-dependent manner [28]. DNA delivery vaccines effectively induce $CD8^+$ T cell responses [29]. Despite T cell activation, it is unclear if neoantigen vaccines can sufficiently lead to tumor rejection [30]. In other experiments, a discrepancy between T cell responses and tumoricidal activity by vaccines using neoantigens has been reported [31][19].

Neoantigen-pulsed dendritic cell vaccines promoted neoantigen-specific T cell frequency in patients with advanced melanoma [32]. Subsequently, a clinical study of a vaccine against melanoma showed that pooled neoantigen candidates immunized with poly ICLC achieved remarkable clinical responses by inducing antigen-specific polyfunctional T cells against tumors [33]. A clinical study using RNA-based vaccines also showed sustained progression-free survival in some patients with melanoma whose neoepitope-specific T cells killed the autologous tumor [34]. In gliomas with typically lower TMB, vaccination using neoantigens generated objective responses, the increase in tumor infiltrating lymphocytes (TILs), or elicitation of epitope-specific T cell responses against peptides [35][36][37]. Recently, there was a successful study on neoantigen vaccines in combination with anti PD-1 antibody treatment. A study showed that neoantigen vaccines elicited neoantigen T cell responses against new ones that had not been included in the antigen of the original vaccine [38]. Not only personalized vaccines, but also off-the-shelf vaccines using neoantigens designed from hot-spot mutations or frameshift mutations have been shown to be safe and feasible [39][40]. Of note, $CD4^+$ T cells are frequently activated by neoantigen vaccines in certain patients as well as in mice preclinical studies. Despite the number of successful examples, it is a fact that not all patients have achieved clinical benefits. The vaccines certainly induce neoantigen-specific T cell responses, but the clinical benefits are limited in the number of patients with cancer. This implies that an infallible selection strategy for neoantigen candidates is required for precision vaccination in future clinics [41][42]. In addition to epitope immunogenicity, patients' T cells, primed by vaccines, need to be evaluated to determine if they can respond to naturally processed tumor neoantigens before using the vaccines.

2.2. Existence of Neoantigen-Specific T Cells in Cancer Patients without Vaccines Treatment

Efficient sampling of tumor-reactive T cells from patients has revealed clinically relevant neoantigen responses. However, it has been reported that low or rare TILs can recognize autologous tumors in ovarian and colorectal cancers [43]. Many solutions have been proposed to overcome the limitations of low availability and low reactivity. Researchers have substituted healthy donors for wide range and robust identification, since there is a risk of underestimation in the use of patient-derived T cells [44]. Despite the limited number of tumor-reactive T cells in patients, it is noteworthy that ICB treatment strongly increased tumor-specific T cells, including neoantigens, in humans and mice [10][45]. Moreover, neoantigen-specific T cells have been identified in TILs from ICB-sensitive tumors compared to ICB-insensitive tumors in a mouse model [10][46]. Additionally, pre-existing neoantigen-specific T cells were reported as a decisive factor for successful immunotherapy outcomes [47]. An enhanced neoantigen immune response in the presence of ICB treatment was more strongly linked to CXCR3 ligands (CXCL9 and CXCL10) than IFN-γ, which enabled sensitive neoantigen detection in a mouse model [48]. To augment immune responses against tumor antigens by ICB treatment, researchers also investigated robust T cell expansion in PD-L1-deleted MC38 tumors, but not parental tumors. Utilizing expanded neoantigen-specific T cells in PD-L1-deficient tumor-bearing mice led to the identification of neoantigens that sufficiently attenuated tumor growth following dendritic cell-based vaccines[49]. Several cell surface expression molecules, such as CD137, PD-1, CD39, and CD134, are used as potential activation markers to detect neoantigen-specific T cells[39][50][51][52]. Analysis of the responses against mutation sequences, peptides, and tandem minigenes can help to identify clinically relevant neoantigens for precision medicine [39][53][54]. Tetramer-based detection and sorting of expanded neoantigen-specific T cells is feasible in both patients and healthy donors [55][56]. With respect to MHC-II neoantigens, $CD4^+$ regulatory T cells (Tregs) in the tumor showed the tumor reactivity, especially for neoantigens. The repertoires of the Treg cells imply the potential target [57]. When patients respond to identified neoantigens, the anti-tumor immune responses are strengthened by these peptide vaccinations. In other cases, neoantigen-specific TCR-T adoptive transfer is expected to be an effective treatment.

3. Neoantigen Candidates as Shared Antigens

Most of the neoantigens are believed to be derived from passenger gene mutations. However, recent progress in human tumor studies has revealed that neoantigens derived from driver gene mutations could generate common and shared neoantigens in certain cases. *IDH1* R132H yields aberrant oncometabolite and induces gliomas, as observed in $CD4^+$ T cells in patients and humanized mice carrying HLA-DRB1*01:01 [58]. This mutation-targeting peptide vaccine could elicit

intratumoral inflammation in most patients harboring multiple HLA alleles [59]. Immunoglobulin-variable regions of lymphoma cells presented on HLA-DR*04:01 are recognized by cytotoxic $CD4^+$ T cells [60]. H3.3 K27M mutation, which results in aberrant gene expression, is the cause of most diffuse intrinsic pontine glioma, and acts as the target of HLA-A2 restricted $CD8^+$ T cells [61]. Frameshift mutant NPM1, which is frequently observed in acute myeloid leukemia, binds to HLA-A*02:01 [62]. Therefore, the TCR from the responding T cells was cloned. Moreover, TP53, a well-known mutated gene in many cancer types, was expressed on HLA-A*02:01 (R175H) and HLA-A*68:01 (R248W) (MHC-I) and HLA-DRB1*13:01 (R175H) and HLA-DRB3*02:02 (Y220C) and HLA-DPB*02:01 (R248W) (MHC-II) [63][64]. TCR against the mutated position of TP53 R175H has already been cloned and validated to recognize many kinds of tumors containing this same mutation [65]. Other famous driver mutations, KRAS G12D and G12V, were recognized by $CD8^+$ and $CD4^+$ T cells, respectively, in the specific alleles [66][67][68]. In addition, the other driver mutant PIK3CA and c-Kit are immunogenic in healthy donors [69]. Driver mutations are necessary to maintain tumor cell characteristics; therefore, more aggressive metastatic pancreatic cancers can harbor uniform gene mutations, thus supporting the shared neoantigens as strong therapeutic targets [70]. Missense and indel mutation-derived neoantigens are not limited, but fusion gene products, typically neighboring joint sequences, have recently been identified as tumor neoantigens even in tumors with low mutation burden tumors [71][72]. Fusion gene products are thought to be frequently involved in tumorigenesis [73]. Hence, fusion genes have become important in the novel neoantigen landscape for immunotherapy as well as driver mutation loci. These studies strongly suggest that NGS mapping should be performed over classical systems solely focusing on the exome to identify neoantigens. Beyond personalized medicine, shared neoantigens can become the primary choice for vaccine targets and neoantigen-specific TCR therapy.

4. Neoantigen Responsiveness and Clonality

4.1. Immunodominant vs. Subdominant Neoantigens

A HLA-A2 restricted Matrix Protein epitope (M1 58-66) of influenza A induces robust T cell responses, whereas the other epitopes elicit weak responses.. The stability or abundance of peptide-HLA complexes, and the frequency or avidity of T cells in recognizing them presumably determine epitope hierarchy [74]. Since T cells responding to immunodominant antigen are spontaneously activated, they can kill target tumor cells. In contrast, subdominant epitopes often hinder reactivity at low levels by immunodominant epitopes, which are difficult to detect. Even if host immunity is sufficient, after evading immune defense against immunodominant epitopes, the subsequent immunity will become weaker than the previous one. Hence, subdominant epitopes are much less suited for tumor eradication in the absence of any treatments, triggering immune-escaped tumor progression. One report showed that subdominant T cell responses yielded incomplete differentiation, skewing to Tc17, and these kinds of T cell activation were evoked by direct vaccinations but not by ICB treatment, indicating the complexity of neoantigen targeting strategy[75]. Although it most likely depends on individual cases, if tumors are stably composed of single clones, complete rejection will be achieved by immunodominant epitopes. However, if they multiply or evolve due to survival, complete rejection becomes difficult. Indeed, the loss of neoantigens is attributed to a reduction in RNA expression, and loss of mutant alleles is observed over the long term in some melanoma patients[76].

4.2. Difference between Clonal Neoantigen and Subclonal Neoantigens

Multi-region analysis of high-grade serous ovarian cancer indicates that immune-selected tumors can evolve in patients with higher TIL density at the tumor interface [77]. Whereas tumor expressing subclonal neoantigens are likely to be killed, the clonal neoantigens in the remaining and proliferated tumor cells are less immunogenic, which leads to aggressive metastasis [78]. This was confirmed by analysis of the early stages of the tumor. In the early stage of non-small cell lung cancer (NSCLC), subclonal neoantigens were retained by a low number of TILs but were eliminated by a high number of TILs. Neoantigens identified in untreated early-stage NSCLC were also less overlapped in progressed tumors from TCGA data. Hence, the low number of TILs at the progressed stages was the result of evasion of the immune response [79]. Accordingly, immunoreactivity is spatially heterogeneous in biopsies from multiple loci of NSCLC, suggesting that analysis of multiple tumor lesions is needed for comprehensive prediction of neoantigen-based therapy [80]. In the experimental model, intratumor heterogeneity also reduced immune responses, which indicates the tumor neoantigen burden in the clonality determines the ICB responses. Hence, an ostensible higher mutation burden due to subclonality is implausible in predicting ICB outcomes[81]. Recently, large-scale meta-analyses of ICB cohort studies clearly demonstrated that clonal TMB was the best predictor of ICB response, followed by total TMB, nonsense-mediated decay escape TMB, indel TMB, and subclonal TMB [82]. Therefore, despite the risk of weakening immunodominant TCRs, targeting multi-neoantigens appears to be cogent for therapeutic use.

Several mechanisms are involved in neoantigen loss. Allelic loss by mutagenesis, chromosome abnormality, and copy number loss by transcriptionally or epigenetically have been identified. A critical factor for immune evasion is the loss of heterozygosity (LOH) of HLA caused by defects in antigen presentation machinery or direct mutation in the HLA complex [83]. The HLA-I genotype determines the ICB responses. Maximal heterozygosity (HLA-A, B, and C) actually improves overall survival after ICB, but also results in some HLA type loss, leading to poor responses [84]. Therefore, immunoediting of various HLA polymorphisms in patients is a key factor for predicting anti-tumor immune responses.

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