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Commentary

DNA hyper methylation editing of T cell receptor signaling and the fellow molecules: a promise strategy of predicting acquired immune checkpoint inhibitors (ICIs) resistance

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Abstract

The promising results of immune checkpoint inhibitors (ICIs) in tumors have changed the current treatment modality for cancer. ICIs response prediction is urgently needed for the personalized therapy approach. In the recent issue of the Journal of Oncology, Zixin Hu et al. proposed that DNA methylation alternations contribute to tumor microenvironment (TME) reshapement to predict the ICIs response. Notwithstanding the global DNA methylation loss, the repression of T cell receptor signaling and the fellow costimulators by DNA hypermethylation contributed to the cold TME and ICIs resistance. Hub immune-associated genes edited by DNA methylation also bonded the driver genes and TME. DNA methylation is a promise predictive biomarker and DNA methylation inhibitors may be a novel strategy to fuel ICIs.

Keywords: DNA methylation, Immune checkpoints inhibitors, Biomarker, T cell receptor, Tumor microenvironment

Introduction

Immune checkpoint inhibitors (ICIs) have yielded great progress in multiple types of cancer, yet the efficacies are not uniform among patients. Therefore, the identification of robust predictive biomarkers of ICIs response is paramount. In a recent paper in the Journal of Oncology [1], the author proposed that hypermethylation and concurrent repression of key genes of T cell receptor (TCR) signaling were associated with cold immunophenotype and ICIs resistance and predicted short overall survival (OS) respectively.

The clinical efficacy of ICIs hinges at least in part upon their ability to unleash the T cells in the tumor microenvironment (TME). Responders exhibit a hot (inflamed) immunophenotype, which is characterized by a preexisting cytotoxic anti-tumor response with dense infiltrated T cells and immune checkpoints leading to immune evasion, whereas non-responders exhibit a cold (non-inflamed) phenotype, which also known as immune-desert and immune-excluded TME and characterized by the absence or exclusion of T cells in the tumor parenchyma [2].

The bridge from the identical DNA sequence (genotype) to disparate gene expression (phenotype) is broadly termed epigenetic regulation, the most widely researched of which is DNA methylation by coordinating with a complex interplay of cis-acting and trans-acting DNA elements. The process promotes the transfer of methyl groups to the 5' site of cytosines in cytosine-guanine (CpG) dinucleotide by DNA methyl transferases (DNMTs). DNA methylation of promoters and gene bodies commonly leads to gene repression.

The growing interest in DNA methylation regulation of TME has been attached. On the one hand, DNA methylation controls the gene regulatory system by activating or repressing the key genes in the immune response signaling to reinvigorate or silence the tumor-infiltrated immune cells [3]. On the other hand, the immune cells are conferred phenotypic stability by DNA methylation to

form the stationary TME [4]. Given the interaction between DNA methylation and TME, incorporating DNA methyl transferase inhibitors (DMNTis) into ICIs synergizes with immunotherapy by potentially alleviating immune evasion [5,6]. The complex crosstalk between DNA methylation and TME is still under exploration. In the commentary, we wish to elucidate the association of DNA methylation and TME and highlight recent progress in this area that may feasibly enhance or preclude ICIs response.

Hot Immunophenotype is Characterized by Global DNA Hypermethylation

In the paper, the author confirmed that the hot TME and patients with a well-response to ICIs displayed as high DNA methylation levels in the lung adenocarcinoma (LUAD). DNA methylation loss accumulates in the cell division and results in the unbalance of gene expression. A past study once reported that DNA demethylation involved in immune evasion increases ICIs resistance by counteracting with high genome instability which induced high tumor mutation load [7]. There is an issue that may be puzzling that the DMNTis which induce the demethylation add the extra value to ICIs while the hot TME and the ICIs sensitive group are displayed as hypermethylation.

The mechanism of epi-immunotherapy has intrigued immunologists and oncologists for years and has inspired and required much experimental ingenuity. DNA methylation alternation is a general cancer hall marker with global methylation loss and methylation of normally unmethylated CpG islands resulting in gene repression. The global CpG island hypermethylation in hot TME might indicate a global trend of loss of protection from methylation accumulation for the high genomic instability and immunogenicity.

In recent years, it gradually become clear that despite the downregulation of global DNA methylation, the key genes of immune response of cold tumors are critically suppressed by DNA hypermethylation. DNMTis increase immunogenicity by demethylation of cancer-testis antigens that are initially suppressed by cytosine methylation in cancer cells. DNMTis also promote the antigen presentation process by restoring human leukocyte antigen (HLA)-class I, β2-microglobulin (β2M), and transporter 1 (TAP1) expression in solid tumors through interferon (IFN) signaling. Moreover, DNMTis can rescue the T cell exhaustion mediated by *de novo* DNA methylation to rewire the TME with the reduced tumor-infiltrating T regulatory cells (Tregs) and increased T-helper type 1 (Th1) cells and $CD8^+$ T cells [6,8].

Repression of Genes in TCR Signaling by DNA Hypermethylation Occurs in the Cold Immunophenotype

For the above problem, the author identified that the hub genes including CD247, CD3D, and lymphocyte-specific protein tyrosine kinase (LCK) in the TCR signaling were down-regulated by the DNA hypermethylation despite the global DNA methylation loss of the non-inflamed TME and ICIs non-sensitive group in LUAD. Among these genes, the silence of CD247, and LCK by hypermethylation were respectively correlated with worse overall survival (OS) [1].

Each T cell is equipped with TCR on the cell surface, which is responsible for the specific recognition of antigens carried by major histocompatibility complex (MHC) molecule. Both the initial activation of naive T cells and the effector phases of T cell-mediated adaptive immune responses are triggered by recognition of the MHC-peptide complex by TCR.

The majority of recirculating T cells participating in adaptive immune response bear αβTCR. The signaling through the TCR is conveyed by a complex of proteins referred to collectively as CD3, which is composed of CD3γε, CD3δε, and CD3ζζ. The cytoplasmic tails of the CD3 molecules are studded with immune tyrosine activation motifs (ITAMs) serving as docking sites for adapter proteins following activation-induced tyrosine phosphorylation [9]. CD247 and CD3D encode ζ and δ chains of CD3 respectively. In the paper, the author proposed a pattern introduced to the immune-desert or immune-exclude phenotype that was characterized by the repression of CD247 and CD3D by DNA hypermethylation to downregulate the signal transduction [1].

The T-cell receptor is non-covalently associated with CD4 or CD8 molecules that also recognize the MHC-peptide antigen. The co-engagement brings the cytoplasmic domains of the TCR-CD3 complex and the respective co-receptor into proximity, enhancing the avidity of the binding and initiating the cascade of intracellular events that activate a T cell. The Src family kinase Lck is associated with the cytoplasmic tails of CD4 or CD8 molecules and is the first tyrosine kinase activated in the T cell signaling. Once the antigen recognition, Lck moves near membrane-associated tyrosine phosphatase to be removed from the inhibitory phosphate group. Reciprocal phosphorylation by nearby Lck molecules at their activating tyrosine sites occurs induces Lck to phosphorylate CD3 ITAM residues [10]. In the non-inflamed immunophenotype, DNA hypermethylation edits the LCK gene to silence the expression of Lck protein, thus inhibiting the initiation of the downstream phosphorylation cascade [1].

Genes Encoding Costimulators on T cells Undergo DNA Hypermethylation in the Cold TME

Generally, the outcome of antigen recognition of T cells is determined by a balance between the engagement of activating and inhibitory receptors. Costimulatory signals enhance the contact between TCR and MHC-peptide and allow the initiation of TCR signaling. Immune checkpoints elicit inhibitory signals to restrict the extreme immune response but lead to immune evasion of the tumor. In the paper, the author declared that the costimulators including CXCR6, intercellular adhesion molecule 3 (ICAM3), and proline-serine-threonine phosphatase interacting protein 1 (PSTPIP1) also bear the reprogramming by DNA hypermethylation in the cold TME.

ICAM-3 is expressed only on naive T cells and is thought to have an important role in the adhesion of T cells to antigen-presenting cells by binding to lymphocyte function associated antigen (LFA-1) expressed on dendritic cells [11]. CXCR6 are critical for the survival and local expansion of effector-like cytotoxic T cells (CTL) in the TME [12]. PSTPIP1 encodes a CD2 cytoplasmic tail-binding protein to orchestrate the F-actin cytoskeleton polymerization of CD4+ T cells which participates in the contact of TCR and MHC-peptide. It was once reported that PSTPIP1 mutation led to T-cell differentiation defects by dysfunction of the immune synapse formation [13].

The past studies demonstrated that DNA hyper methylation on promoters of CD28, cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed death-1 (PD-1) led to the downregulation of the

corresponding genes and dysfunction of effective immune response which were linked to the ICIs failure [14]. CD28 expressed on T cell surface is the best defined costimulators for TCR signaling, which is the principal costimulatory receptor for delivery of second signals for T cell activation and binds the costimulatory molecules B7-1 (CD80) and B7-2 (CD86) expressed on the surface of activated APCs. CTLA-4 and PD-1 counteract with the CD28 signal to inhibit the TCR signaling and are best established their physiologic role in self-tolerance. Researchers also identified that DNA hyper methylation of chemokines such as CX3CL1 induced a deficiency of T cells in TME [15].

Driver Gene Crosstalk with TME by the Bridge of DNA Methylation

Mutations of key driver genes involves cancer genesis and tumor evolution. The epigenetic modulation such as DNA methylation act as a bridge of genotype and phenotype and are attributed to the further adaptation and transformation of tumor with the driving of oncogenes. DNA methylation supplement oncodrivers for silencing promoters of tumor suppressors. Oncogenes such as TP53 promote the formation of epigenetic instability by elevating the global DNA methylation [16]. In the paper, the author declared that a potential mechanism of epidermal growth factor receptor (EGFR) mutations in LUAD modifying the TME with the bridge of DNA methylation of serglycin (SRGN).

According to the public data of TCGA and GEO, SRGN is repressed by DNA hyper methylation in the EGFR mutated (EGFR mut) samples compared with the EGFR wild type (EGFR wt). Patients with EGFR wt and high expressed SRGN displayed as an inflamed TME with activated antigen presentation process, whereas EGFR mut patients with low expressed SRGN was characterized as a non-inflamed phenotype. SRGN of ICIs non-responders tended to be low expressed, which indicated that SRGN might be an immunological biomarker. Furthermore, DNA hyper methylation of SRGN and low expression SRGN predict longer OS.

SRGN encodes a kind of proteoglycan secreted by the extracellular matrix of tumor cells and increases the epithelial-mesenchymal transition to enhance the migratory capacity and increase the resistance to apoptosis. Moreover, SRGN create a pro-inflammatory TME and is regarded as a driving factor of aggressive phenotype [17]. EGFR mut patients were always excluded for the potential resistance to ICIs in the past clinical trials of ICIs, while the ICIs response of EGFR wt patients are determined by the heterogeneity of tumor immune feature. The study identified SRGN as the predictive biomarker of immunophenotype and ICIs response. Expression of SRGN may be the bond of EGFR type and TME in LUAD. As SRGN is closely regulated by DNA methylation, it can be a potential epigenetic target to enhance the ICIs response.

Conclusion

DNA methylation involved in stabilizing repression of gene transcription and modulate the fate of immune cell populations in TME. Despite the global DNA methylation loss, the hub genes of immune signaling repression by DNA hyper methylation contributed to the cold TME. In the paper, Zixin Hu et al. identified that silence of CD247, CD3D, and LCK by DNA methylation in the TCR signaling and the TCR costimulators such as CXCR6, ICAM3, and PSTPIP1 contributes to cold immunophenotype and ICIs resistance in LUAD patients. Among these genes, low expression and hyper methylation of CD247, LCK, and PSTPIP1 predicted short OS. Moreover, DNA methylation act as a bonding role of driver genes and TME. The author declared that repression of SRGN by DNA hyper methylation occurred in the EGFR mut LUAD. Patients with EGFR wt and high expressed SRGN were linked with the hot TME and better ICIs response. Given that the modification of DNA methylation of TME account for prediction of ICIs response and concomitant medication of DNMTis and ICIs is promise.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

ZX-H and HJ-C contributed to the idea and design. HJ-C gave her idea about the DNA methylation and immunotherapy. ZX-H consulted the literature and accomplished the manuscript writing and revision. All authors have read and approved the submitted version.

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Ethical Approval Statement

The study was carried out with the ethical approval of the institutional Ethics Committee of the Faculty of Medicine at China-Japan Friendship Hospital approval (2021-122-K80). Publicly available data was used in the article, so the ethical approval was waived.

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