Editorial

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Immunotherapy

Immune modulation via epigenetic targeting to overcome immune checkpoint inhibitor resistance

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Immunotherapy has revolutionized anticancer therapy over the last decade, with impressive responses seen in many patients and six immune checkpoint inhibitors (ICIs) now approved by the US FDA for the treatment of solid organ malignancies [1]. The best responses and survival benefits are seen in melanoma patients, with approximately 60% of patients treated with combination immunotherapy (anti-CTLA4 and anti-PD-1 inhibition) still alive at 3 years [2]. However, many patients with advanced solid organ malignancies have suboptimal responses or acquire resistance relatively quickly. There is therefore a real clinical imperative to find ways to optimize immunotherapy and overcome ICI resistance with intense research interest in this area.

Resistance to ICIs can be either intrinsic or acquired after an initial response. Although the mechanisms are complex, most ICI resistance appears to be related to alterations in innate or adaptive immunity. For instance, impaired-dendritic cell maturation or *B2M* mutations render antigen presentation defective and impair T-cell activation [3]. Some tumors lack sufficient neoantigen load or mutational burden, PD-L1 expression or the necessary interferon signature to drive specific T-cell activation [3]. Cancer cells can evolve to modify the tumor microenvironment to one that is hostile to effector T cells via various mechanisms including downregulation of chemokines, upregulation of PD-L1, induction of resistance pathways like IDO and CD73, mutations in *PTEN* and *JAK1/2*, activation of alternative immune checkpoints, and upregulation of immunosuppressive cells [3].

As a result, current strategies to overcome resistance are similarly diverse but include: increasing DNA damage/neoantigens with chemotherapeutic agents, PARP inhibitors or radiotherapy; blocking potentially resistant pathways; blocking inhibitory signals (LAG3, TIM3, VISTA and BTLA); activating stimulatory pathways like OX40 and ICOS; vaccines; administration of antigen-specific engineered T cells; and epigenetic manipulation [4].

However, many of these strategies have disappointed in practice. Despite promising preclinical data, combination approaches have yet to show benefit in Phase III clinical trials. For instance, an inhibitor of IDO (epacadostat), an immunosuppressive enzyme that suppresses effector T cells and NK cells and activates T-regulatory cells (Tregs) and myeloid-derived suppressor cells (MDSCs), failed to show any additional benefit when combined with the PD-1 inhibitor pembrolizumab over pembrolizumab alone in metastatic melanoma [5]. Strategies that block intracellular signaling pathways are often compensated for by another pathway, since intracellular tumor pathways are not linear but in fact extraordinarily complex interrelated signaling networks. For example, BRAF blockade in melanoma is overcome by reactivation of the MAPK pathway, PI3K/Akt pathway activation, or genetic or epigenetic gain or loss of genes such as *NRAS*, *PTEN* or *AKT* [6]. In this way, single target inhibition in one pathway often activates

Future Medicine

an alternate tumor-promoting pathway or results in the development of new mutations that curtail therapeutic responses.

Epigenetic therapies, however, may overcome some of these limitations because they manipulate reversible changes in the tumor; they can overcome immune and nonimmune mechanisms simultaneously. Epigenetics is often defined as heritable alterations in transcription without changes to the DNA sequence, classically as methylation, acetylation, phosphorylation and ubiquitination of histones to regulate chromatin conformation or DNA methylation to regulate transcription. However, while epigenetic drugs in principle exert their action at the chromatin level, it is becoming increasingly clear that their effects are broader via modulating nonhistone proteins, particularly transcription factors and co-activator proteins. For example, LSD1, an epigenetic enzyme overexpressed in various cancers and associated with poor patient prognosis and originally thought to exclusively demethylate lysine residues on histones, is now known to demethylate a range of important cancer-causing nonhistone proteins including DNMT1, STAT3 and HSP90 [7]. Furthermore, emerging evidence implicates epigenetic changes in a broad range of immune processes implicated in ICI resistance including immune memory cells, CD8 T-cell activation, T-cell exhaustion, PD-L1 expression, and the regulation of immune suppressor cells like Tregs and MDSCs. These broad immune and nonimmune effects of epigenetic enzymes and the drugs that block them are important in the context of overcoming ICI resistance in anticancer therapy.

T-cell exhaustion or reduced T-cell activation is crucial for tumor cell immune evasion. Qin *et al*. demonstrated an inverse correlation between LSD1 expression and expression of the chemokines CCl5, CXCL9, and CXCL10, and LSD1 inhibition enhanced H3K4me2 in the promotor regions of these genes and reactivated $CD8^+$ T cells [8]. In another study, LSD1 loss enhanced antitumor T-cell immunity, tumor immunogenicity and T-cell infiltration [9]. At the same time, LSD1 knockdown upregulated PD-L1, a negative regulator of T cells, which rendered the tumor cells responsive to anti-PD-1 therapy [9]. LSD1 inhibition also activates type-1 interferon via inhibition of RISC and increased endogenous retroviral expression. Changes in chemokines and upregulation of PD-L1 may potentially prime the tumor for ICIs. Indeed, PD-L1 and LSD1 inhibition was more effective than either agent alone *in vivo*. Furthermore, other epigenetic drugs like HDAC inhibitors upregulate PD-L1 and PD-L2 on tumor cells and the expression of CTLA4 and PD-1 in tumor-infiltrating lymphocytes [10].

Immune suppressor cells have emerged as key regulators of antitumor immunity over the last decade. MDSCs are granulocytic cells found in the tumor microenvironment associated with higher stage cancer and poor survival; they protect the tumor from immune responses, including immunotherapy. MDSCs also promote Tregs, tumor-associated macrophages and cancer-associated fibroblasts (CAFs). HDAC inhibition has shown promising results in preclinical studies in reducing Tregs and MDSCs and consequently inhibiting primary tumor growth and metastasis [11]. Downregulation of $FoxP3^+$ Tregs and tumor shrinkage was consistently observed in number of preclinical epigenetics studies, especially with H3K27me3 inhibition [12]. Similarly, there is evidence to suggest that DNA methylation is important for the maintenance of CAFs and can be reversed with DNMT1 inhibitors [13].

Tumor-associated antigens (TAA) are essential for the immune recognition of cancer cells, and epigenetic manipulation can enhance TAA expression. For example, cancer testis antigen is a well characterised TAA that is repressed in some solid organ cancers, thereby suppressing the immune response, and cancer testis antigen expression is augmented by DNMT and HDAC inhibitors [14]. Furthermore, HDAC inhibitors can upregulate surface antigen presenting molecules including MHC, TAP-1, TAP-2 and other co-stimulatory molecules like CD40, CD80 and ICAM-1 [15]. Epigenetic drugs may also contribute to tumor immunogenicity by affecting DNA damage responses and apoptosis. LSD1 inhibits apoptosis by repressing the transcriptional activity of p53, so LSD1 inhibition could potentially enhance neoantigen production [7].

One of the unique features of at least some epigenetic drugs is their anticancer properties against cancer stem cells (CSCs). CSCs are subset of cancer cells which are highly tumorigenic, invasive and resistant to standard anticancer therapy so are implicated in tumor initiation, resistance and progression. There is no direct evidence of the impact of immunotherapy on CSCs; however, indirect evidence suggests that they are probably unaffected by ICIs. Resistant signatures, including genes that control epithelial to mesenchymal transition (EMT), are found in ICI nonresponsive patients. These tumors were enriched for EMT and TGF-β signaling genes [16]. EMT leads to loss of cell adhesion molecules and gain of a mesenchymal phenotype, a fundamental process for the development of CSCs, in which TGF-β signaling plays an important role. In addition, overexpression of stem cell markers like NANOG, SOX2, OCT4 and KLF and the secretion of chemokines and cytokines leads to immune escape. High levels of IL-4 were found in CSCs from colorectal cancer patients, leading to weak immunogenicity and T-cell exhaustion [17]. CD44, a common CSC surface marker, appears to be associated with low immunogenicity [18].

Epigenetic manipulation has been shown to eradicate CSCs effectively in various preclinical studies, especially with LSD1 and HDAC inhibitors [19,20].

In conclusion, epigenetic drugs are emerging in their own right as effective anticancer agents and as attractive adjuncts to ICIs to enhance immune responses in several ways including by modifying key regulatory cytokines, effector T-cell responses and immune suppressor cells. Epigenetic drugs can also target elements of the tumor that promote metastasis and resistance such as CSCs. Although we have a basic understanding of epigenetic alterations *in vitro*, further work is required to understand the *in vivo* effects of epigenetic drugs due to their likely effects not only on chromatin and histones, but also nuclear and cytosolic pathways, different tumor compartments, and noncoding RNAs. Identifying specific epigenetic signatures and understanding *in vivo* mechanisms of action will ultimately be necessary for appropriate patient selection and choice of appropriate drug combinations.

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