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Natural Killers out of Thin Air

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The regulatory mechanisms controlling natural killer (NK) cells in the tumor microenvironment remain unknown. In this issue, Ni. et al. provide evidence that the transcription factor HIF-1α acts as a key negative regulator of NK cell metabolic fitness in the tumor microenvironment that critically impedes NK cell anti-tumor immune responses.

Natural killer (NK) cells are recognized as key immune effector cells in cancer immunosurveillance by their ability to recognize and eliminate malignant transformed cells without major histocompatibility complex (MHC) restriction or prior sensitization. In addition to immunosurveillance against carcinogenesis, NK-cell-mediated cytotoxicity plays a key role in the elimination of disseminated tumor cells as observed in metastatic solid tumors and hematological malignancies. There is limited evidence to support that NK cells effectively exert tumor cytotoxicity in established solid malignancies, although a recent study showed that NK cells indirectly support the optimal efficacy of anti-programmed cell death-1 (PD-1) blockade by crosstalk with intratumor dendritic cells (Barry et al., 2018). While substantial advances have been made to understand immune checkpoint molecules and soluble inhibitory factors (e.g., cytokine transforming growth factor β [TGF-β], adenosine) that regulate tumor immunity, many additional and related factors potentially impede and exclude NK-cell-mediated antitumor immunity in the established solid tumor microenvironment. In this issue of *Immunity*, Ni et al. (2020) demonstrated that hypoxia-inducible factor 1 α (Hif-1α) is a key molecular switch that dampens NK-cell-mediated antitumor immunity, providing novel insight into immune regulation of NK cells in the hypoxic tumor microenvironment.

The generation of a hypoxic tumor microenvironment is a key hallmark of advanced malignancies, and growing evidence supports the link between hypoxia and immunosuppression in the tumor microenvironment (Noman et al., 2015). HIF-1 was first discovered by Gregg L. Semenza and his colleagues in 1991 as a transcriptional activator of erythropoietin gene in response to hypoxia, and now HIF-1α has been recognized as a master regulator of oxygen homeostasis by its ability to activate more than 100 target genes related with the adaption to hypoxic stress, such as angiogenesis, erythropoiesis, glucose metabolism, and cell survival (Fallah and Rini, 2019). To understand the role of Hif-1α in NK cells, Ni et al. generated mice with NKp46^{+} cell-specific deletion of Hif-1α (namely, *Hif1a*$^{f/f}$; *Ncr1*^{iCreTg} mice), and these mice were subcutaneously challenged with RMAs thymoma (MHC class I-negative tumor cells due to TAP deficiency). Strikingly, deletion of Hif-1α in NK cells showed an increased expression of effector molecules including the cytokine interferon-γ (IFN-γ), leading to remarkable tumor control *in vivo*. By contrast, there was no difference in controlling lung metastases between *Hif1a*$^{f/f}$; *Ncr1*^{iCreTg} mice and control mice, suggesting that the negative regulation of NK cells by Hif-1α predominantly occurred in larger established tumor masses. Of note, using the same conditional gene-targeting approach, Krzywinska et al. previously showed that NK-cell-specific deletion of Hif-1α inhibited the growth of MC38 colon tumor by inducing vascular endothelial growth factor (VEGF)-dependent, aberrant non-productive angiogenesis (Krzywinska et al., 2017). By contrast, Ni et al. showed that neutralization of VEGF did not alter the tumor control in *Hif1a*$^{f/f}$; *Ncr1*^{iCreTg} mice, suggesting the anti-tumor phenotypes were independent of angiogenesis. The discrepancy might be explained by the different sensitivity to NK-cell-mediated cytotoxicity and/or differential dependency on functional neo-vasculature between the two tumor models. However, results from both studies highlighted that Hif-1α induced the dramatic reprogramming of NK cells in the tumor microenvironment (Ni et al., 2020; Krzywinska et al., 2017).

To characterize the Hif-1α-mediated transcriptional reprogramming of NK cells, Ni et al. performed single-cell RNA sequencing of tumor-infiltrating NK cells isolated from *Hif1a*$^{f/f}$; *Ncr1*^{iCreTg} mice and control wild-type mice. Unsupervised clustering revealed a NK cell subset with high expression levels of genes encoding IFN-γ and CD69, which were predominantly observed in Hif-1α-deficient NK cells. Pathway analysis showed a clear enrichment of the NF-κB pathway and pathways related to NF-κB activities in Hif-1α-deficient NK cells, suggesting that Hif-1α might negatively regulate proinflammatory responses that lead to the activation of NK cells.

To identify a key factor that might drive the NF-κB-mediated activation, Ni et al. performed transcriptional profiles of cytokines in whole-tumor tissues and found the upregulation of IFN-γ and interleukin 18 (IL-18) in tumor tissues isolated from *Hif1a*$^{f/f}$; *Ncr1*^{iCreTg} mice compared to control mice. Given that IL-18 is known to induce IFN-γ by activating the IL-18 receptor (IL-18R)-MyD88-NF-κB signaling pathway, Ni et al. hypothesized that IL-18 was predominantly responsible for activated phenotypes in Hif-1α-deficient NK cells. While there was no difference in protein levels of IL-18 in tumor tissues, Ni et al. found that the IL-18R signaling pathway was highly enriched in Hif-1α-deficient NK cells compared to WT NK cells, indicating that deletion of Hif-1α could confer hyper-responsiveness to IL-18 in NK cells. Indeed, neutralization of IL-18 abrogated antitumor phenotypes and downregulated the activation of NF-κB in Hif-1α-deficient NK cells. Moreover, Hif-1α-deficient NK

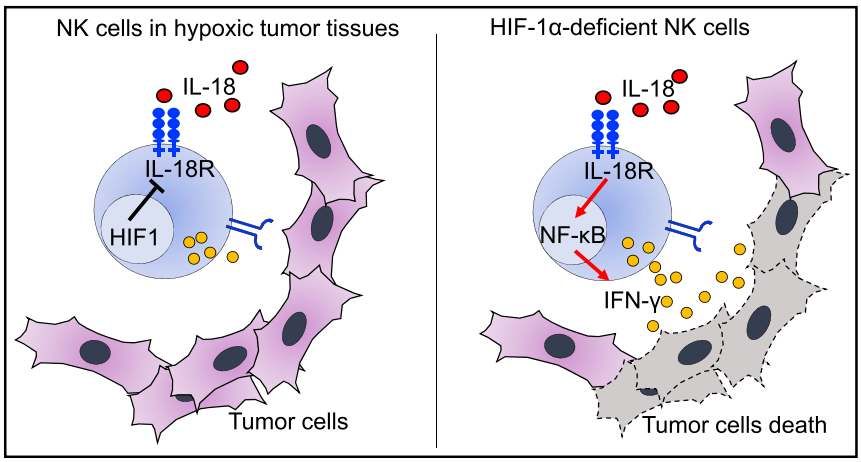

Figure 1. HIF-1a Is a Key Immune Checkpoint of NK Cells
The expression of HIF1 critically impedes antitumor immunity by inhibiting the IL-18-dependent activation of NK cells (left). Unleashing the HIF-1-mediated negative regulation of NK cells leads to improved tumor control by IL-18-driven IFN-γ (right).

cells showed higher production of IFN-γ in response to IL-12 and IL-18 under hypoxic conditions, which was associated with an improved oxygen consumption rate (Ni et al., 2020). These results demonstrated that HIF-1α acted as a key negative regulator of the IL-18-dependent activation and metabolic fitness of NK cells in the tumor microenvironment.

Lastly, and perhaps most importantly, Ni et al. characterized the relationship between *HIF1A* expression and the *NK-IL18-IFNG* signature in human cancers. Strikingly, there was an inverse correlation between the expression of *HIF1A* and the *NK-IL18-IFNG* signature in NK cells from non-small-cell lung carcinoma tissues, and tumor-infiltrating NK cells showed higher levels of *HIF1A* expression with lower *NK-IL18-IFNG* signatures compared to peri-tumor NK cells. These results further supported that HIF-1α dampened NK cell activities in the hypoxic core of tumor tissues. Moreover, Ni et al. showed that the *NK-IL18-IFNG*^{high} signature predicted better prognosis in multiple cancer types, including skin cutaneous melanoma, breast cancer, and cervical carcinoma (Ni et al., 2020). Overall, Ni et al. provided robust evidence that HIF-1α was a key immune checkpoint molecule in tumor-infiltrating NK cells, which critically limited IL-18-driven NK cell antitumor responses (Figure 1).

Tumor hypoxia orchestrates immunosuppression by multiple mechanisms, including enhancement of immunosuppressive activities in tumor-associated macrophages, generation of immunosuppressive adenosine, and inducing functional impairment of effector lymphocytes (Noman et al., 2015). It is noteworthy that deletion of Hif-1α in NK cells alone could dramatically improve antitumor immunity despite the presence of other hypoxia-associated immunosuppressive pathways. Recently, another group reported that hypoxic tumor microenvironment triggers mitochondrial fragmentation of NK cells, which functionally and metabolically dampens NK-cell-mediated antitumor immunity (Zheng et al., 2019). Together, hypoxic stress response and reprogramming might be a major barrier for NK cell activities in the tumor microenvironment. Results from Ni et al. strongly supported that the IL-18R signaling pathway was the key target of HIF-1-mediated regulation. As hypoxic stress can trigger the release of damage-associated molecular pattern molecules (DAMPs), which stimulate innate inflammatory responses (Nakamura and Smyth, 2017), the hypoxic core of tumor tissues might be characterized as an IL-18-rich milieu. In this context, releasing HIF-1α-mediated negative regulation of IL-18R signaling might confer significant functional advantages to NK cells. Further studies are warranted to understand the spatiotemporal interplay among DAMPs, IL-18, and NK cells in the hypoxic tumor microenvironment.

While this study by Ni et al. clearly demonstrated that deletion of Hif-1α augmented NK cell activities, the molecular mechanism of hypoxia-induced reprogramming of NK cells remains largely unknown. It was reported that a combination of hypoxia, TGF-β1, and a demethylating agent converted peripheral blood NK cells into a decidual NK-cell-like phenotype characterized by reduced cytotoxicity and high pro-angiogenic capability (Cerdeira et al., 2013). Moreover, Gotthardt et al. showed that NK-cell-specific deletion of VEGF-A improved tumor control (Gotthardt et al., 2016). These results raise a possibility that hypoxia-adapted NK cells might acquire pro-tumor functions, and thus, it is important to understand whether targeting HIF-1α can redirect hypoxia-adapted NK cells into antitumor effector cells. The functional and phenotypic plasticity of NK cells governed by HIF-1α requires further investigation.

Based on the fact that high expression levels of HIF-1 are associated with poor prognosis in a wide range of cancers, various therapeutic approaches targeting HIF have been developed either by direct inhibition of HIF-1 expression and/or function or by indirect inhibition by blocking upstream or downstream pathways (Fallah and Rini, 2019). Although lack of specificity and potency remains a major challenge to therapeutically target HIF against cancer, most clinical trials have been focused on clinical efficacies either by monotherapy or in combination with chemotherapy. Targeting HIF-1 might be able to harness antitumor immunity by effector lymphocytes, including but not limited to NK cells, and further studies are warranted to safely and effectively target HIF-1 in combination with immunotherapy. Alternatively, given that anti-CD19 chimeric antigen receptor (CAR) NK cell therapy has shown promising clinical responses in non-Hodgkin's lymphoma patients in a recent clinical trial (Liu et al., 2020), gene targeting of HIF-1 in CAR-NK cells might be a feasible approach. Overall, this work by Ni et al. reveals a novel immune regulatory mechanism of NK cells in the tumor microenvironment that has broad implications in immunobiology and clinical immunotherapy.

DECLARATION OF INTERESTS

M.J.S. has research agreements with Bristol Myers Squibb and Tizona Therapeutics and is a member

of the Scientific Advisory Board (SAB) for Tizona Therapeutics and Compass Therapeutics.

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Genetics Meets Epigenetics in Treg Cells and Autoimmunity

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Most autoimmunity-associated SNPs in the genome map to noncoding regulatory regions in T cells, but the nature of underlying epigenetic mechanisms and any normal purpose in T cell differentiation remain unclear. In this issue of *Immunity*, Ohkura et al. establish that crucial SNPs linked to autoimmune disease are enriched in DNA regions of CpG demethylation that govern Treg cell development and function.

A profound shift in understanding the basis of disease-associated genetic alterations occurred with the publication of ENCODE mapping of gene regulatory regions and the revelation that single nucleotide polymorphisms (SNPs) associated with disease commonly occur outside of coding genes (ENCODE Project Consortium, 2012). In the field of autoimmune disease, the influence of SNPs in gene regulation in T cells has been noted (Farh et al., 2015), but a deep understanding has not been achieved, and specific mechanisms are not known. In fact, T cell development displays particularly complex gene regulation involving numerous differentiation pathways, and these lineages require intricate orchestration via epigenetic regulation. One striking aspect of T cell activity is the vital balance between "too much" and "too little" immune function: failure to achieve the appropriate balance can manifest in autoimmune disease or immune deficiency, respectively. Autoimmunity is a worldwide disorder implicated in numerous common diseases, such as type 1 diabetes and rheumatoid arthritis. While mutations are well documented in genes encoding important T cell transcription factors and cell-surface proteins, many SNPs occur outside of genes and are presumably involved in epigenetic regulation. A major question here is whether autoimmunity results from regulatory SNPs leading to, on the one hand, overactivity of conventional effector T cells (Tconv) whose job is to respond to infection or, on the other hand, underactivity of regulatory T (Treg) cells whose job is to suppress exuberant immune activity. A second important issue is whether the SNPs in autoimmune disorders lead to a a precise mechanistic defect in a particular epigenetic regulatory pathway. In this issue of *Immunity*, Ohkura et al. (2020) investigate autoimmune-associated SNPs, linking them to a DNA demethylation signature of active genes in Treg cells, whereas DNA methylation is maintained at these genes in Tconv cells (Figure 1).

The process of cell differentiation is characterized by formation of epigenetic landscapes and transcriptional programs to control the fate of specific cell types.