“Suffocating” tumors by blocking adaptation to hypoxia: a new headway in melanoma immunotherapy

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ABSTRACT

We recently reported that inhibiting Hypoxia-inducible Factor-1α (Hif1α) transcriptional activity improves melanoma immunotherapy by driving immune cells into the tumor microenvironment (TME). This Author’s View provides additional perspectives on how hypoxia inhibitors combined with immunotherapy can be used as innovative approaches to improve the therapeutic benefit of melanoma patients.

Given its role in tumor resistance to therapies, hypoxia is an important target in cancer treatment. However, deploying hypoxia inhibitors in the clinic requires a full understanding of hypoxia signaling. After 30 years of research on the mechanisms by which cells adapt to hypoxia, William G. Kaelin Jr., Sir Peter J. Ratcliffe, and Gregg L. Semenza have been awarded the Nobel Prize in Medicine 2019. Their seminal discoveries have contributed to the development of strategies to target hypoxia in tumors using hypoxia-activated prodrugs or blocking the transcription activity of hypoxia-inducible factors (HIFs).

The wealth of clinical and preclinical data accumulated over a decade underlined the central role of hypoxia in impairing the efficacy of cancer immunotherapy. Hypoxia modulates the immune landscape of tumors and harms the tumor-killing functions of immune cells. Depending on the hypoxia grade, and regardless of their presence in the tumor microenvironment (TME), immune cells are unable to fulfill their cytotoxic function and may be corrupted to support tumor growth. Therefore, developing hypoxia inhibitors has inspired significant interest in combination with immunotherapy. The ultimate aim of such a combination is to extend the therapeutic benefit of cancer immunotherapy approaches to a large number of cancer patients. Indeed, clinical data showed that the remarkable benefit of immune checkpoint blockade (ICB)-based immunotherapy was only observed in a low proportion (30%) of melanoma patients, while the majority of patients have a short-term benefit or no benefit at all. Although the low response rate to ICB is not clearly attributed to hypoxia, it should be highlighted that melanoma contains large regions of hypoxia and anoxia (estimated to be up to 50–60%). Hypoxia contributes to many aspects of melanoma development and progression by inducing melanocyte transformation and increasing the heterogeneity of melanoma cell populations in a HIF1A-dependent manner. Moreover, hypoxia resulted in the activation of signaling pathways involved in cancer cell invasiveness and epithelial-to-mesenchymal transition (EMT). Furthermore, hypoxia directly or by inducing metabolic shifts affects the function and the trafficking of immune cells to the TME. Therefore, there is a pressing need to better understand how hypoxia affects antitumor immunity and how it can be manipulated to improve the treatment outcome of immunotherapy.

We investigated the impact of targeting the transcriptional activity of Hif1α on improving the immunotherapy benefit in a melanoma mouse model. By CRISPR/Cas9, we deleted in Hif1α the domain responsible for its interaction with Hypoxia-inducible Factor-1β (Arnt) and generated B16-F10 melanoma cells expressing truncated Hif1α (termed Del-Hif1α) unable to form a heterodimer with Arnt. We showed that Del-Hif1α is transcriptionally inactive under hypoxia in B16-F10 cells and corresponding tumors.

In the immunocompetent, but not in immunocompromised, there was a significant reduction of tumor growth and weight as well as improvement of survival in mice bearing Del Hif1α tumors compared to those bearing full-length (Fl) Hif1α tumors. These data highlight that blocking the transcription activity of Hif1α impacts tumor development by involving the host immune system. Profiling the immune landscape and the chemokine network of tumors revealed a significant increase in the infiltration of...
Figure 1. Targeting the transcription activity of Hif1a recruits immune cells to the melanoma tumor microenvironment. a: Melanoma tumors are characterized by the presence of poorly oxygenated areas [Oxygen pressure (pO₂) less than 8 mmHg]. These hypoxic areas result from an imbalance between low O₂ supply due to an abnormal vascularization and high O₂ consumption by tumor cells. Hypoxic areas are poorly infiltrated by cytotoxic immune cells. b: Under a low pO₂ or hypoxic microenvironment, the enzymatic activity of prolyl hydroxylase domain-2 (Egln1/Phd2) protein is inhibited. Such inhibition leads to blocking the prolyl hydroxylation (OH) of Hif1a, inhibition of both Hif1a-dependent ubiquitination (Ub) and Von Hippel-Lindau (Vhl) interaction, and subsequent proteasomal degradation of Hif1a (B1). Consequently, Hif1a accumulates in the cytoplasm, translocates to the nucleus, and forms a heterodimer with hypoxia-inducible Factor-1β (Arnt). The Hif1a/Arnt complex binds to the hypoxia-responsive element (HRE) and activates transcription of several downstream target genes (B2). c: Inhibiting the transcription activity of Hif1a can be achieved by deleting the domain responsible for the formation of a heterodimer with Arnt using CRISPR/Cas9 gene-editing technology (C1). In hypoxic cells expressing deleted Del-Hif1a, the formation of heterodimer Hif1a/Arnt is prevented, and the transcription activity of Hif1a is blocked. In these cells, the expression of the pro-inflammatory (C-C motif) ligand 5 chemokine (Ccl5) is activated by a mechanism that is not fully understood. The release of Ccl5 by tumor cells expressing Del-Hif1a increases the recruitment of cytotoxic immune cells in the microenvironment, which subsequently release of (C-C motif) ligand 2 chemokine (Ccl2) to support the establishment of inflammatory signature in melanoma (C2).
of CD45+, Natural killer (NK), CD4+, and CD8+ cells into Del-Hif1α tumors compared to Fl-Hif1α tumors. Such infiltration was associated with an increased release of pro-inflammatory (C-C motif) ligand 5 and 2 chemokines (Ccl5 and Ccl2) in the TME of Del-Hif1α tumors (Figure 1).

Although much remains to be learned about the mechanism(s) responsible for the infiltration of immune cells into Hif1α-defective tumors, we believe that analyzing the quality and integrity of the blood microvascular network could provide more insight into how immune cells have infiltrated the TME. Nevertheless, our data revealed that inhibiting the transcriptional activity of Hif1α would enhance the therapeutic benefit of immunotherapy in melanoma. To provide the experimental evidence of this concept, we used acriflavine, reported as a drug suppressing the transcriptional activity of Hif1α by preventing Hif1α/Arnt dimerization, hence mimicking our B16-F10 melanoma model. While monotherapy based on anti-PD-1 or TRP-2 vaccination has no or moderate impact on tumor growth, combining acriflavine with both anti-PD-1 and TRP-2 vaccination suppressed B16-F10 tumor growth, presumably by releasing Ccl5 and Ccl2 involved in driving cytotoxic immune cells to the TME. The translational aspect of our study is underscored by data generated from melanoma patients described in the TCGA database. Compared to patients having high hypoxia scores, those exhibiting low hypoxia scores express a high level of CCL5, which correlates with increased expression of NK, C3, CD4, and CD8 cell markers and improved survival.8

The expression of Hif1α is closely correlated with glycolytic products (pyruvate, lactate).9 Under hypoxia, inhibiting the Mif (Macrophage Migratory inhibition Factor)/Cd74 axis that reduces lactate production in Cta4-resistant melanoma cells significantly reduces the expression of Hif1α.10 Therefore, it would be interesting to evaluate the expression of glycolytic products in the microenvironment of Del-Hif1α tumors and assess the signaling pathway of Mif/Cd74. In this context, we believe that inhibitors of the Mif/Cd74 pathway can be used as hypoxia modulators to improve the benefit or restore the sensitivity to Cta4-based cancer immunotherapy in melanoma.

Overall, our results highlight the critical role of hypoxia in the establishment of immune-suppressive TME. We believe the hypoxic status of tumors should be considered in immunotherapy protocols in the clinic. This study will contribute to designing an innovative combination with hypoxia inhibitors that might create tremendous enthusiasm in melanoma therapy.

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References
1. Noman MZ, Janji B, Kaminska B, Van Moer K, Pierson S, Przanowski P, Baert S, Berchem G, Romero P, Mami-Chouaib F, et al. Blocking hypoxia-induced autophagy in tumors restores cytotoxic T-cell activity and promotes regression. Cancer Res. 2011;71(18):5976–5986. doi:10.1158/0008-5472.CAN-11-1094.
2. Chouaib S, Noman MZ, Kosmatopoulos K, Curran MA. Hypoxic stress: obstacles and opportunities for innovative immunotherapy of cancer. Oncogene. 2017;36:439–445. doi:10.1038/onc.2016.225.
3. Vaupel P, Mayer A. Hypoxia in cancer: significance and impact on clinical outcome. Cancer Metastasis Rev. 2007;26:225–239. doi:10.1007/s10555-007-9055-1.
4. Bedogni B, Welford SM, Cassarino DS, Nickoloff BJ, Giaccia AJ, Powell MB. The hypoxic microenvironment of the skin contributes to Akt-mediated melanocyte transformation. Cancer Cell. 2005;8:443–454. doi:10.1016/j.ccr.2005.11.005.
5. Widmer DS, Hoek KS, Cheng PF, Eichhoff OM, Biedermann T, Raaijmakers MG, Hemmi S, Dummer R, Levesque MP. Hypoxia contributes to melanoma heterogeneity by triggering HIF1alpha-dependent phenotype switching. J Invest Dermatol. 2013;133:2436–2443. doi:10.1038/jid.2013.115.
6. You L, Wu W, Wang X, Fang L, Adam V, Nepomiva E, Wu Q, Kuca K. The role of hypoxia-inducible factor 1 in tumor immunoevasion. Med Res Rev. 2021;41(3):1622–1643. doi:10.1002/med.21771.
7. Noman MZ, Parpal S, Van Moer K, Xiao M, Yu Y, Arakelian T, Viklund J, De Milito A, Hasmim M, Andersson M, et al. Inhibition of Vps34 reprograms cold into hot inflamed tumors and improves anti-PD-1/PD-L1 immunotherapy. Sci Adv. 2020;6:eaca7881. doi:10.1126/sciadv.aaca7881.
8. Lequeux A, Noman MZ, Xiao M, Van Moer K, Hasmim M, Benoit A, Bosseler M, Viry E, Arakelian T, Berchem G, et al. Targeting HIF-1 alpha transcriptional activity drives cytotoxic immune effector cells into melanoma and improves combination immunotherapy. Oncogene. 2021;40(28):4725–4735. doi:10.1038/s41388-021-01846-x.
9. Meijer TW, Kaanders JH, Span PN, Bussink J. Targeting hypoxia, HIF-1, and tumor glucose metabolism to improve radiotherapy efficacy. Clin Cancer Res. 2012;18(20):5585–5594. doi:10.1158/1078-0432.CCR-12-0858.
10. de Azevedo RA, Shoshan E, Whang S, Markel G, Jaiswal AR, Liu A, Curran MA, Travassos LR, Bar-Eli M. MIF inhibition as a strategy for overcoming resistance to immune checkpoint blockade therapy in melanoma. Oncoimmunology. 2020;9(1):1846915. doi:10.1080/2162402X.2020.1846915.