MELANOMA LYMPH NODE METASTASES ARE ASSOCIATED WITH HYPOXIA AND IMMUNOLOGIC DYSFUNCTION

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Background Deranged cellular metabolism is a hallmark of cancer and the hypoxic tumor microenvironment (TME) is associated with T-cell dysfunction. We have shown that in melanoma patient samples, high tumor cell oxidative stress is associated with T-cell dysfunction, intratumoral hypoxia, and worse clinical outcomes. Less is known about the impact of tumor cells on the environment of sentinel lymph nodes (SLN), though recent studies have implicated SLN as critical sites in the efficacy of the anti-tumor immune response. Thus, we sought to assess the impact of melanoma SLN metastasis on intra-nodal immune cell composition and hypoxia.

Methods We identified 87 patients with primary melanoma who had undergone SLN biopsy. Tumor FFPE slides were stained and imaged using Vectra OPAL panels (immune: CD3, FOXP3, CD8, CD68; tumor: SOX10; checkpoint receptor/ligands: PD-1, PD-L1, IDO [indoleamine 2,3-dioxygenase]; hypoxia: HIF-1a [Hypoxia-inducible factor 1-alpha], CAIX [carbonic anhydrase IX]). 732 regions of interest (ROIs) were selected from 47 patients who had a positive SLN, and 426 ROIs from 40 patients with a negative SLN. 20X images were analyzed for cell segmentation by StarDist, and phenotyping by Random Forests machine learning models. Images from adjacent tumor sections were co-registered using SimpleITK to enable spatial evaluation of markers across panels. Cell density and distances in tumor, tumor/stroma boundary (+/-50um), and stroma were compared between groups using linear mixed-effects models (LMM). The study was approved by the University of Pittsburgh’s Institutional Review Board.

Results Within tumor infiltrated sentinel lymph nodes, areas of SOX10+ metastatic melanoma were enriched for CAIX, a marker of hypoxia. IDO, an immunosuppressive enzyme which is upregulated in hypoxic conditions, was more frequently expressed within the surrounding stroma (CD3-SOX10-) of tumor infiltrated nodes compared to negative nodes (p<0.0001). The immune composition of CD68+ myeloid cells, FOXP3+ T-cells, and CD8+ T-cells was similar in infiltrated and non-infiltrated nodes. Consistent with other reports of immune dysfunction within tumor infiltrated nodes, the frequency of PD-1+CD8+ cells was significantly enriched compared to negative lymph nodes (p<0.01).

Conclusions Infiltration of tumor draining lymph nodes by metastatic melanoma is associated with markers of immunologic dysfunction and intranodal hypoxia. This constellation of features recapitulates the hypoxic primary tumor microenvironment. Studies are underway to evaluate the potential of oxidative balance within melanoma SLN as a prognostic biomarker.

Ethics Approval The study was approved by the University of Pittsburgh’s Institutional Review Board.