TERTIARY LYMPHOID STRUCTURES (TLS) IN DESMOPLASTIC MELANOMAS (DM) DIFFER FROM NON-DM-ASSOCIATED TLS BY THEIR INTRATUMORAL LOCATION AND ENHANCED IMMUNE ACTIVITY

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Background Tertiary lymphoid structures (TLS) are ectopic lymphoid organs that are localized near tumors and other sites of inflammation, and are commonly believed to support anti-tumor immunity. We previously published studies that show that most desmoplastic melanomas contain TLS, and that TLS in cutaneous metastatic melanomas varied widely in maturation state, in proportions of proliferating T and B cells, and in markers of B cell function, including AID and CD21. Thus, we hypothesized that there may be diversity in TLS function, or immunologic activity, among melanomas. To address this hypothesis, we evaluated TLS in primary desmoplastic melanomas (DM), and non-desmoplastic melanomas (non-DM) for markers of cell proliferation which are indicative of early immune activity.

Methods DM and non-DM tumor specimens, which included primary melanomas (PM), and cutaneous metastatic melanomas (CMM), were evaluated for TLS by multiplex Immunofluorescence histology, by staining for CD20, CD8, PNA⁺, Ki67, FoxP3, and DAPI. Lymphoid aggregates were identified in 20x spectrally unmixed images by visual inspection and identified as TLS if possessing organized T-cell and B-cell regions in addition to high endothelial venule-like vasculature (PNA⁺). TLS were identified in 30 out of 64 screened (48%) CMM, 4/4 non-DM PM, and 8 out of 11 screened (73%) DM. Immune cells localized in TLS were enumerated using Halo software (Indica Labs). Mann-Whitney tests were used for statistical assessments.

Results DM commonly contain a dense network of fibroblasts and associated stroma, which are not typical for other non-DM (PM and CMM). TLS in DM are located throughout the tumors, intratumorally, in sharp distinction from the peritumoral location of TLS in non-DM. Furthermore, when compared to TLS of non-DM (PM and CMM), TLS of DM contain increased densities of CD20⁺ B cells (PM $p=0.007$; CMM $p<0.0001$) and CD8⁺ T cells (PM $p=0.017$; CMM $p=0.0006$), and a higher proportion of proliferating (Ki67⁺) CD20⁺ B cells (PM $p=0.04$; CMM $p=0.009$).

Conclusions Recently published studies have identified tumor-associated fibroblasts as the likely initiating cells for TLS formation in murine melanomas. The intratumoral location of TLS in DM puts them in close proximity to the dense fibroblasts and desmoplastic stroma in these tumors, which may be responsible for their intratumoral location. The increased density of B and T cells, and higher proportion of proliferating (Ki67⁺) B cells, in DM than in non-DM, suggests that there may be greater immune activation, increased germinal center maturation, or less regulation in TLS of DM.

Ethics Approval Approval was obtained for these studies under IRB protocol #'s 10598 and 19694.

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