waves of gene expression in GC B cells in HNSCC tumors, ultimately revealing a novel transitional state for GC B cells in the tumor microenvironment (TME).
Conclusions Understanding B cell function in human cancers and how different TMEs influence B cells and TLS are important for devising novel therapeutic options for cancer patients. Ultimately, development of therapeutics to enhance B cell responses in the TME should be prioritized as a compliment to T-cell mediated therapies.

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537 CONVENTIONAL TYPE 1 DENDRITIC CELLS AND NATURAL KILLER CELLS DEMONSTRATE STRONG CORRELATION TO T LYMPHOCYTE INFILTRATION IN CERVICAL CANCER TUMORS

[1]Anushka Dikshit*, [2]Xiao-jun Ma, [2]Bingqing Zhang. [1]*Advanced Cell Diagnostics, Newark, CA, USA*; [2]*Advanced Cell Diagnostics, Bio-Techne, Newark, CA, USA*

Background The ability of T cells to mediate anti-tumor immunity has been harnessed to develop successful immunotherapies in recent years. Although direct presentation of tumor antigens by tumor cells plays an important role in the effector function of cytotoxic T lymphocytes (CTLs), cross-presentation by professional antigen presenting cells such as dendritic cells (DCs) is vital for priming naive CD8+ T cells and developing a sustainable cytotoxic response. Natural killer (NK) cells within the tumor microenvironment (TME) recruit a specific population of DCs called conventional type 1 DCs (cDC1s) into the TME by secreting chemokines such as CCL5 and XCL1. However, these cells are very low in abundance and are characterized by the expression of numerous markers, making their detection in the tissue context challenging.
Methods Therefore, to interrogate the presence of cDC1 and NK cells in the TME and reveal their spatial relationship we utilized the highly sensitive and specific RNAscope Multiplex Fluorescence in situ hybridization (ISH) assay. Probes for XCR1, THBD, CLEC9A, and CCR5 were used to identify cDC1 cells within 4 cervical cancer tumors. These tumors were then assessed for the presence of NK cells by using specific marker probes such as CD56 and NCR1 and chemokines XCL1 and CCL5. Finally, CTLs were visualized to determine if there is a correlation between the presence of cDC1 and NK cells and CTL infiltration within the cervical cancer tumors.
Results Our results revealed a strong correlation between the presence of NK cells, cDC1 cells, and CTLs within 3 out of 4 cervical cancer samples. The NK cells showed expression of the chemokines XCL1 and CCL5, which are the ligands for XCR1 and CCR5 respectively, suggesting that the XCR1+/CCR5+ cDC1 cells may have been potentially recruited by these NK cells. Regions high in cDC1 and NK cells also showed significantly higher levels of CTL recruitment, as indicated by the presence of CD8+/IFNG+ T cells. Conversely, 1 of the 4 cervical cancer samples demonstrated relatively lower levels of NK cells which correlated with lower cDC1 cells and in turn lower CTL infiltration.
Conclusions In conclusion, by utilizing the RNAscope Multiplex ISH assay we were able to identify and visualize the spatial relationship between NK cells, CTLs, and cDC1 cells, a rare subset of DC cells that excel at presenting tumor antigens to T cells. Using this technology, it is possible to spatially interrogate the TME and detect specialized immune cells when assessing response to immunotherapies.

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538 HARNESSING CROSS-DRESSING DENDRITIC CELLS TO STRENGTHEN ANTI-TUMOR IMMUNITY

Ellen Duong*, Timothy Fessenden, Arjun Bhutkar, Stefani Spranger. *Koch Institute, Cambridge, MA, USA*

Background Cytotoxic ($CD8^+$) T-cells are required for tumor eradication and durable anti-tumor immunity.[1] The induction of tumor-reactive $CD8^+$ T-cells is predominately attributed to a subset of dendritic cells (DC) called Batf3-driven DC1, given their robust ability to cross-present antigens for T-cell priming and their role in effector T-cell recruitment.[2–4] Presence of the DC1 signature in tumors correlates with improved survival and response to immunotherapies.[5–7] Yet, most tumors with a DC1 infiltrate still progress, suggesting that while DC1 can initiate tumor-reactive $CD8^+$ T-cell responses, they are unable to sustain them. Therefore, there is a critical need to identify and engage additional stimulatory DC subsets to strengthen anti-tumor immunity and boost immunotherapy responses.
Methods To identify DC subsets that drive poly-functional $CD8^+$ T-cell responses, we compared the DC infiltrate of a spontaneously regressing tumor with a progressing tumor. Multicolor flow immunophenotyping and single-cell RNA-sequencing were used to profile the DC compartment of both tumors. IFNγ-ELISpot was performed on splenocytes to assess for systemic tumor-reactive T-cell responses. Sorted DC subsets from tumors were co-cultured with TCR-transgenic T-cells ex vivo to evaluate their stimulatory capacity. Cross-dressing (in vivo/ex vivo) was assayed by staining for transfer of tumor-derived $H\text{-}2^b$ MHC complexes to Balb/c DC, which express the $H\text{-}2^d$ haplotype. Protective systemic immunity was assayed via contralateral flank tumor outgrowth experiments.
Results Regressor tumors were infiltrated with more cross-presenting DC1 than progressor tumors. However, tumor-reactive $CD8^+$ T-cell responses and tumor control were preserved in $Batf3^{-/-}$ mice lacking DC1, indicating that anti-tumor immune responses could be induced independent of DC1. Through functional assays, we established that anti-tumor immunity against regressor tumors required $CD11c^+$ DC and cGAS/STING-independent type-I-interferon-sensing. Single-cell RNA-sequencing of the immune infiltrate of regressor tumors revealed a novel $CD11b^+$ DC subset expressing an interferon-stimulated gene signature (ISG^+ DC). Flow studies demonstrated that ISG^+ DC were more enriched in regressor tumors than progressor tumors. We showed that ISG^+ DC could activate $CD8^+$ T-cells by cross-dressing with tumor-derived peptide-MHC complexes, thereby bypassing the requirement for cross-presentation to initiate $CD8^+$ T-cell-driven immunity. ISG^+ DC highly expressed cytosolic dsRNA sensors (RIG-I/MDA5) and could be therapeutically harnessed by exogenous addition of a dsRNA analog to drive protective $CD8^+$ T-cell responses in DC1-deficient mice.
Conclusions The DC infiltrate in tumors can dictate the strength of anti-tumor immunity. Harnessing multiple stimulatory DC subsets, such as cross-presenting DC1 and cross-dressing ISG^+ DC, provides a therapeutic opportunity to enhance anti-tumor immunity and increase immunotherapy responses.

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539 HIGH DIMENSIONAL ANALYSIS OF THE HUMAN LYMPH NODE DURING MELANOMA PROGRESSION REVEALS SHIFTS IN MYELOID CONTENT THAT RELATE TO DIFFERENTIAL T CELL CONTENT

[1]Anastasia Prokopi*, [1]Marta Lopez-Gonzalez, [1]Jan Verhoeff, [1]Rieneke Van de Ven, [1]Juan Garcia-Vallejo, [2]Patrizia Stoitzner, [1]Tanja de Gruijl. *[1]Amsterdam UMC, Amsterdam, Netherlands; [2]Medical University of Innsbruck, Innsbruck, Austria*

Background The sentinel lymph node (SLN) in melanoma represents the crossroads of the initiation of effector T cell responses and of lymphatic metastasis of the primary tumor. As such, alterations in the human LN immune cell network during melanoma progression are of particular interest for the development of effective immunotherapeutic approaches for each stage of disease.

Methods We used mass cytometry (CyTOF) and multiparameter flow cytometry to characterize the alterations in the major immune populations in the human LN. We included LN derived from healthy donors (n=10), tumor-negative (SLN-, n=7) and tumor-positive SLN (SLN+, n=3) and LN metastatic samples (n=4).

Results Our results show that melanoma progression in the LN is accompanied by increased relative frequencies of myeloid cells, B cells and NK cells whereas T cell rates are significantly decreased. More specifically, for the myeloid cells we observed a decrease in frequencies of migratory cDC subsets and of LN-resident cDC and macrophage subsets in the SLN accompanying early melanoma development and metastasis. In fully metastatic LN from patients with advanced melanoma, a clear predominance of inflammatory, monocyte-derived subsets was observed. Simultaneously with this shift in myeloid subsets, an increase in CD4+ Tregs and CD8+ effector T cell subsets became apparent with metastatic progression in the LN. Both Tregs and CD8+ effector T cells in LN metastases were further characterized by relatively high expression of PD-1 and TIGIT immune checkpoints.

Conclusions The changes observed in the myeloid compartment accompanying metastatic progression in melanoma-draining LN, were found to be related to the shifts in lymphocytic subsets and their differentiation and activation state. Our results provide insights into the steady-state immune characteristics of the healthy human LN and identify all the changes that accompany melanoma progression through the different stages and give important clues about possible therapeutic interventions, aiming at immune potentiation of the SLN.

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540 TRANSCRIPTIONALLY DEFINED IMMUNE LANDSCAPE IN HUMAN GLIOMAS

Pravesh Gupta*, Minghao Dang, Krishna Bojja, Huma Shehwana, Tuan Tran, Linghua Wang, Krishna Bhat. *The University of Texas MD Anderson Cancer Center, Houston, TX, USA*

Background Brain immunity is largely myeloid cell dominated rather than lymphoid cells in healthy and diseased state including malignancies of glial origins called as gliomas. Despite this skewed myeloid centric immune contexture, immune checkpoint and T cell based therapeutic modalities are generalizably pursued in gliomas ignoring the following facts i) T cells are sparse in tumor brain ii) glioma patients are lymphopenic iii) gliomas harbor abundant and highly complex myeloid cell repertoire. We recognized these paradoxes pertaining to fundamental understanding of constituent immune cells and their functional states in the tumor immune microenvironment (TIME) of gliomas, which remains elusive beyond a priori cell types and/or states.

Methods To dissect the TIME in gliomas, we performed single-cell RNA-sequencing on ~123,000 tumor-derived sorted CD45+ leukocytes from fifteen genomically classified patients comprising IDH-mutant primary (IMP; n=4), IDH-mutant recurrent (IMR; n=4), IDH-wild type primary (IWP; n=3), or IDH-wild type recurrent (IWR; n=4) gliomas (hereafter referred as glioma subtypes) and two non-glioma brains (NGBs) as controls.

Results Unsupervised clustering analyses delineated predominant 34-myeloid cell clusters (~75%) over 28-lymphoid cell clusters (~25%) reflecting enormous heterogeneity within and across glioma subtypes. The glioma immune diversity spanned functionally imprinted phagocytic, antigen-presenting, hypoxia, angiogenesis and, tumoricidal myeloid to classical cytotoxic lymphoid subpopulations. Specifically, IDH-mutant gliomas were predominantly enriched for brain-resident microglial subpopulations in contrast to enriched bone barrow-derived infiltrates in IDH-wild type especially in a recurrent setting. Microglia attrition in IWP and IWR gliomas were concomitant with invading monocyte-derived cells with semblance to dendritic cell and macrophage like transcriptomic features. Additionally, microglial functional diversification was noted with disease severity and mostly converged to inflammatory states in IWR gliomas. Beyond dendritic cells, multiple antigen-presenting cellular states expanded with glioma severity especially in IWP and IWR gliomas. Furthermore, we noted differential microglia and dendritic cell inherent antigen presentation axis viz, osteopontin, and classical HLAs in IDH subtypes and, glioma-wide non-PD1 checkpoints associations in T cells like Galectin9 and Tim-3. As a general utility, our immune cell deconvolution approach with single-cell-matched bulk RNA sequencing data faithfully resolved 58-cell states which provides glioma specific immune reference for digital cytometry application to genomics datasets.

Conclusions Altogether, we identified prognosticator immune cell-signatures from TCGA cohorts as one of many potential immune responsiveness applications of the curated signatures for basic and translational immune-genomics efforts. Thus, we not only provide an unprecedented insight of glioma TIME