ABSRACT BOOK FlowTex 2018

A rapid differential diagnostic strategy in CD45 negative neoplasms by validating a novel four color flow panel

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Background: In order to accomplish a rapid differential diagnosis of non-hematological neoplasms, a novel four color flow cytometric panel was designed with cellular markers of CD45, EPCAM, CD56, and CD138.

Materials and methods: One hundred and two samples were obtained from patients who were suspected of non-hematological neoplasms from 2014 to 2017 in hematology department of Houston Methodist Hospital. After regular eight color flow cytometric panel screening, all samples were additionally evaluated by the novel four color pattern of CD45-FITC/CD138-PE/EPCAM-Percp-Cy5.5/CD56-APC.

Results: With the help of different expression patterns of this four color panel, we could make a preliminary differential diagnosis of a series of neoplasms and identify some normal clusters of cells. These findings were as follow: 1) Pattern CD45+EPCAM-CD56+CD138- represented a NK cell phenotype; 2) Pattern CD45+EPCAM-CD56+CD138+ represented a malignant myeloma phenotype; 3) Pattern CD45+EPCAM-CD56-CD138+ represented a plasma cell or malignant myeloma phenotype, or even a B cell non-Hodgkin lymphoma phenotype; 4) Pattern CD45dim+EPCAM-CD56+CD138- represented an AML phenotype with aberrant CD56 expression; 5) Pattern CD45dim+EPCAM-CD56+/-CD138+ represented a malignant myeloma phenotype, and also represents a plasmablastic lymphoma or lymphoma with plasma differentiation phenotype; 6) Pattern CD45-EPCAM+CD56+CD138-/+ mostly represented a small cell carcinoma or carcinoma with neuroendocrine differentiation phenotype; 7) Pattern CD45-EPCAM+CD56-CD138-/+ represented an epithelial cell phenotype, and also most carcinoma and adenocarcinoma exhibited this phenotype; 8) Pattern CD45-EPCAM-CD56+CD138-/+ represented a series of diseases, including in malignant myeloma, small cell carcinomas, small cell carcinomas or carcinomas with neuroendocrine differentiation, malignant melanoma and sarcoma, also mesothelial cells exhibited this phenotype; 9) Pattern CD45-EPCAM-CD56-CD138+ mostly represented a malignant myeloma phenotype.

Conclusion: Surprisingly we found this simple four color panel could distinguish a serious of diseases, including in some normal cell phenotype, malignant myeloma and a large portion of non-hematological neoplasms. Followed a diagnostic strategy upon the different expression patterns of this novel four color flow panel, we could make a preliminary differential diagnosis of non-hematological. This make it possible for pathologists to utilize more targeted antibodies of ICH and test treatment for different kinds of diseases, which could economize time and cost for patients to get a more accurate final diagnose. Importantly, therapies could be started early especially for patients with acute and severe disease statuses.

KEY WORDS: Non-hematological neoplasm, EPCAM, CD56, CD138, CD45

Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade

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Background: Immune checkpoint blockade is able to achieve durable responses in a subset of patients, however despite such significant clinical progress we still lack a fundamental understanding of the mechanisms of anti-CTLA-4 and anti-PD-1 induced tumor immune rejection. CTLA-4 and PD-1 regulate T cell activation through different molecular and cellular mechanisms and act at different stages of T cell activation. As such, we hypothesized that anti-CTLA-4 and anti-PD-1 checkpoint blockade induce tumor rejection through distinct cellular mechanisms.

Materials and methods: To address this hypothesis, we utilized mass cytometry which enables characterization of more than 40 parameters at single cell resolution and unsupervised cellular classification. Using this approach, we comprehensively profiled the immune infiltrates of MC38 and B16BL6 murine tumors from mice treated with anti-CTLA-4, anti-PD-1, or control antibodies. We also performed similar analyses of surgically resected melanomas from patients being treated with checkpoint blockade therapy.

Results: In both tumor models, more than 13 distinct tumor infiltrating T cell populations were identified. Both anti-CTLA-4 and anti-PD-1 checkpoint blockade modulated the frequencies of only a subset of these tumor infiltrating T cell populations. Furthermore, of multiple exhausted-like CD8 T cell populations identified, the frequencies of only two subsets correlate with outcome, suggestive of functional heterogeneity within phenotypically exhausted T cells. Most notably, we find that anti-CTLA-4, but not anti-PD-1, modulates the CD4 effector compartment by inducing the expansion of Th1-like CD4 effector T cells. Observations from mass cytometry analyses of surgically resected melanoma tumors from patients being treated with anti-CTLA-4 or anti-PD-1 therapy were consistent with these preclinical findings.

Next we utilized similar methodologies to investigate the cellular mechanism of combination anti-CTLA-4 and anti-PD-1 therapy. Although combination therapy largely enhanced effects observed in monotherapies, combination therapy differentially modulated specific exhausted-like CD8 T cell populations. These data suggest that combination therapy modulates T cell function and mediates tumor rejection through mechanisms that are in part distinct from either monotherapy.

Conclusions: Our findings indicate that anti-CTLA-4 and anti-PD-1 modulate specific tumor infiltrating T cell populations and utilize distinct cellular mechanisms to induce tumor rejection. Anti-CTLA-4, but not anti-PD-1, induces expansion of CD4 effector T cells. Furthermore, these data suggest that combination therapy modulates T cell function differently than monotherapies. These findings have implications for the rational design of combinatorial therapeutic approaches and expand our understanding of the mechanisms that regulate T cell activity.

Acknowledgements: We acknowledge MDACC core facility NCI Support Grant P30CA16672.

Computational Evaluation of Immune Signatures by Mass Cytometry for Cancer Immunotherapy Clinical Trials

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Blockade of immune checkpoint receptors in immune cells has proven to be remarkably effective in the treatment of multiple cancer types. However, despite the therapeutic success of cancer immunotherapy, not all patients respond satisfactorily and adverse effects might be developed. It is then necessary to identify cellular biomarkers that can predict clinical outcome or toxicities, through characterization of circulating immune cells and those in the tumor microenvironment. Mass cytometry has become the method of choice for cell phenotyping, enabling high-dimensional, single-cell analysis of protein abundance, without the interference from fluorophore spectral overlap seen by conventional flow cytometry. The increase in the number of measurable parameters has brought the need for automated computational tools to visualize cytometry findings in high dimensional space. In this work, we described the pipeline used at the Immunotherapy Platform at MDACC to identify immune signatures for cancer immunotherapy clinical trials. We evaluated a combination of different computational algorithms, including visualization tools (viSNE, Giphi), clustering tools (Scaffold, SPADE, Phenograph, FlowSOM) and other tools (Citrus, CellCnn, Qlucore) in cytof datasets. We integrated multiple computational algorithms to construct a reproducible and robust analysis pipeline and improve our ability to identify cell subsets associated with clinical responses. Our pipeline includes quality control, data normalization, preprocessing, transformation, dimension reduction, clustering, visualization and statistical evaluation. We have applied this analysis strategy in different clinical trials and it has proven to be a powerful tool to profile multiple markers across cell types and to define clusters of interest with potential to predict clinical outcome in cancer patients treated with immunotherapy.

A Cell-Based Platform to Monitor Activation of the Unfolded Protein Response

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Protein biosynthesis needs and secretory demands in mammalian cells are constantly integrated and coordinated within the endoplasmic reticulum (ER), a complex and highly dynamic network of membranous tubules that host protein folding and provides a fundamental checkpoint for the processing of native proteins through the secretory pathway. A number of intracellular and extracellular stimuli alter protein folding in the ER and lead to impairment of ER protein homeostasis and ER stress. Not surprisingly, ER stress characterizes the cellular pathogenesis of a variety of human diseases, primarily neurodegenerative diseases. The cellular response to ER stress manifests through activation of complex transcriptional and post-translational regulatory programs collectively referred to as the unfolded protein response (UPR). By responding to changes in the protein folding status of the ER, the UPR thus provides a sensor of cellular homeostasis, a notion that points to the need for robust and reliable tools to monitor activation of the UPR. To respond to this need, we developed a cell-based platform to monitor the UPR signaling pathways upon ER stress stimuli in a high-throughput setup using flow cytometry. Using CRISPR-Cas9 technology, we generated fluorescent reporter cell lines engineered to link the expression of a reporter to the expression of representative genes of the three UPR signaling pathways (ATF6, IRE1, PERK). Because the fluorescent reporter gene is integrated in the chromosome and gene expression is linked to that of the endogenous UPR gene, the fluorescence of these cell lines recapitulates with high fidelity the transcriptional and translational events that regulate the UPR. We obtained preliminary data validating the use of the UPR reporter cell lines to monitor UPR activation and evaluate the different temporal activation of the UPR signaling pathways generated by distinct ER stress stimuli. By combining the use of genome-edited cell lines and flow cytometry, we developed a system to monitor the UPR signaling pathways. Results from this project will provide a platform to map ER-stress inducing stimuli with the specific nature of the UPR response and UPR outcome. This platform will help decipher the role of the UPR in a variety of physiological and pathological processes.