B-CELL RECEPTOR (BCR) REPERTOIRE AND ANTIBODY RECOGNITION ANALYSIS OF MELANOMA PATIENTS TREATED WITH NEOADJUVANT IMMUNE CHECKPOINT BLOCKADE (ICB)

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Background Enriched B cell signature is correlated with positive outcome of immune checkpoint blockade (ICB) therapy in melanoma1 and other cancers.2 B cells have the capability to engage and activate other immune effector cells by recognition and further presentation of tumor antigens. B cells can also secrete an array of cytokines that modulate tumor microenvironment (TME) driving anti-tumor response. Though the mechanism through which B cells contribute to the treatment outcome is still not completely understood, we hypothesize that the BCR and antibodies produced by tumor-residing B cells play an important role in immunotherapy response by recognizing tumor.

Methods Immunoglobulin sequences from intratumoral B cells were reconstructed from single-cell RNAseq data using VDJ Puzzle algorithm.3 Additionally, single B cells were sorted from melanoma tumor tissue and/or adjacent lymph nodes. DNA sequences encoding antibody heavy and light chains were recovered by RT-PCR. Reconstructed immunoglobulin sequences were annotated and analyzed using NCBI IgBlast4 or IMGT/HighV-QUEST.5 The hallmarks of antigen recognition like somatic hypermutation (SHM), isotype switching, and clonal expansion were analyzed on heavy chain sequences. Simultaneously, selected Ig transcripts were cloned into expression vectors enabling the production of tumor-derived recombinant monoclonal antibodies (rmAbs) in mammalian expression system. Antibodies were subsequently purified and tested for tumor and/or cell line binding. A subset of which are being subjected to antigen identification analysis.

Results We successfully utilized our workflow pipeline (figure 1) to generate human rmAbs from tumors of 10 melanoma patients; 6 responders, 2 partial responders and 2 nonresponders. In total, we have been cloning hundreds of antibodies, over 600 from complete and partial responders, and almost a hundred from nonresponders. We observed expansion of selected clones and acquisition of SHM in a manner consistent with an immune selection process. We find a large number of antibodies bind tumor tissue or related cell lines and antigen identification is ongoing.

Conclusions Ig sequence analysis indicates that the B cells in TME participate in antigen-driven selection process and a large percentage of the antibodies recognize antigens from the tumor or related cell lines. We conclude that the expanded B cell signature is recognizing and being selected for by tumor antigens. The utilization of single-cell RNAseq and single-cell Ig isolation in patients with response to ICB therapy can be used to identify and clone antibodies associated with tumor samples. This method has potential to identify unique tumor antigens and offer novel therapies.

REFERENCES

Ethics Approval These patients were treated at the University of Texas MD Anderson Cancer Center and had tumor samples collected and analyzed under Institutional Review Board (IRB)-approved protocols (2015-0041, 2012-0846, LAB00-063 and PA17-0261).

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A section of a tumor is digested and stained with specific antibodies to identify subsets of B cells. Ig transcripts are recovered from single-sorted cells via RNAseq or RT-PCR. Variable domains of heavy and light chains are either synthesized or amplified and cloned into expression vectors for subsequent expression in mammalian cells. Figure created with BioRender.com