Commentary

New applications for deep sequencing of the T cell receptor repertoire in cancer patients

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Glioblastoma (GBM) is the most frequent and aggressive primary brain tumor arising in the central nervous system. In the past several years, the emergence of new immunotherapeutic options for the treatment of GBM has resulted in a need to better understand the breadth and diversity of the tumor-specific αβ T populations. The discovery of more sensitive biomarkers or peripheral blood immune monitoring assays could potentially result in more accurate methods to predict the forms of treatments a particular patient may respond best to, thus saving valuable time and potentially resulting in better clinical outcomes. One potential avenue to study the tumor-infiltrating lymphocyte (TIL) population in GBM is to use high throughput sequencing. As an example of this, Sims *et al.* (1), sequenced the T cell populations from low and high grade glioma samples, non-neoplastic tissue, and peripheral blood and quantified the diversity and differences that existed in and between these populations. In addition to that, the authors reported a detectable signature in peripheral blood that correlates with low TIL divergence, which may have future clinical significance.

The ability of a T cell to recognize and attack a tumor cell is dependent on its ability to recognize a peptide antigen complexed within the major histocompatibility complex (MHC) of the tumor cell through its unique T-cell receptor, an α/β heterodimeric receptor consisting of two polypeptide chains each with a variable and constant domain. To generate the capability to recognize a wide range of existing antigens, each developing T cell in the body undergoes somatic recombination of the V and J (and sometimes D) gene loci to yield a unique T cell receptor (TCR) sequence. Embedded in the variable section of the sequence is the complementarity-determining region 3 (CDR3) domain, the portion of the sequence responsible for interacting with and recognizing the peptide-MHC complex. Upon recognition of an antigen, the T cell undergoes clonal expansion to rapidly produce multiple clones of itself. A tumor-specific TIL population would presumably be comprised of various sub-populations from different T-cell clones that have undergone clonal expansion and have multiple TCRs that recognize distinct antigens on GBM cells.

To study the TIL population and its differences to other T-cell populations, Sims *et al.* isolated total RNA from low and high-grade human glioma samples, non-neoplastic then tissue, and patient matched peripheral blood samples, and reversed transcribed the RNA to create α- and β-chain TCRseq libraries. For each patient, the libraries were sequenced and different combinations of CDR3 amino acid sequences and VJ cassette combinations were characterized.

First, the authors examined the diversity of the TIL TCR repertoire by calculating the Shannon entropy, a numerical value that measures the diversity of the TCR repertoire with higher values representing greater diversity. Noting that the CDR3 region was comprised of sequences from the V and J gene regions (V-J dependent component) and those not from those regions (V-J independent component), the authors sought to determine how each component contributed to the overall diversity of the population. Their results showed that the V-J dependent component contributed more to the diversity of the TIL population, but when compared to TILs from non-neoplastic tissue, the diversity in the glioma TIL population from the V-J independent component was greater than the diversity from the non-neoplastic V-J independent component. Having examined the diversity of the TIL population in the glioma samples, the authors next examined whether a quantifiable difference existed between the TIL and PBMC population. To do this, the authors calculated the Jensen-Shannon (JS) divergence, which uses the entropy values of two groups to calculate whether a difference exists between the groups.

Examining the V-J independent component, the authors found that little difference existed between the PBMC and non-neoplastic populations, but when these two groups were compared to the glioma TIL population, a quantifiable difference arose. However, interestingly, when the lower grade and higher grade glioma groups were compared, the TILs from the lower grade glioma group exhibited higher diversity than the TILs from the higher grade.

Finally, the authors looked to see whether CDR3 sequences of the peripheral T-cells contained a predictive signature for TIL and PBMC divergence in glioma patients. Using the most frequent amino acid CDR3 motifs in the peripheral blood, the authors clustered the population of T cells based off the presence or absence of these motifs. From this clustering, the authors hypothesized that there would be a correlation between the presence of these common CDR3 motifs and diversity in the TIL population. When examined, the authors found an inverse correlation of diversity between the cluster of peripheral T cells with the common CDR3 motifs and the glioma TILs. From this, the authors proposed that the presence of these common CDR3 motifs in a patient's PBMC sample represents a signature that can be noninvasively identified and used to predict low TIL divergence in the glioma.

The recent findings by Sims *et al.*, build on other exciting applications of next generation sequencing to survey the TCR repertoire in cancer patients, particularly to study the effects of immunotherapy on T-cell diversity. For instance, Robert *et al.* (2), used next generation sequencing to study the peripheral T cell population of melanoma patients after CTLA-4 blockage and found increased T cell diversity in the blood after treatment. Our group has also used this technology to examine the TCR repertoire in human glioma and peripheral blood samples after treatment with autologous tumor lysate-pulsed DC vaccination (3). We found that greater TCR overlap (the percentage of unique TCRβ sequences shared between peripheral blood and tumor) before or after DC vaccination treatment correlated with better survival, which offers a predictive tool when assessing how well a patient may respond to treatment. In the future, we hope to apply TCR sequencing to isolate and study shared TCR sequences between *ex vivo* expanded TILs and glioma-derived TIL DNA to find which T-cell clones are the most relevant to an immune-mediated antitumor response. Regardless of the application of this technology, the ability to study the T cell population by sequencing the TCR repertoire points to exciting new and innovative methods of sensitively measuring T cell responses in cancer patients.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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