RON KINASE AS POTENTIAL IMMUNOTHERAPEUTIC TARGET FOR AUGMENTING T CELL ACTIVITY IN BREAST CANCER METASTASIS

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Background Metastatic breast cancer is the overwhelming cause of mortality among all breast cancers. Harnessing the immune system to eliminate metastasis is appealing because it does not require knowledge of the “drivers” of heterogeneous tumors or specific targeted-therapies. T cells play critical roles in anti-tumor immunity, but they are often dysfunctional in cancer patients. How T cells become dysfunctional during tumor progression is still controversial and requires further investigation. Our lab identified host expression of the receptor tyrosine kinase Ron as a potential target for breast cancer immunotherapy. We discovered that a specific Ron isoform, short-form Ron (sfRon), plays a key role in regulating the anti-tumor immune response. Importantly, genetic deletion of sfRon (Ron SF-/-) in the mouse host nearly eliminates outgrowth of established lung micro-metastases through potent activation of T cell-mediated anti-tumor immune responses. We sought to test the hypothesis that sfRon expression in T cells regulates their function and that inhibiting Ron kinase activity is a viable therapeutic strategy to augment T cell activity against metastasis.

Methods To investigate sfRon in the suppression of anti-tumor immunity, we conducted single-cell RNA sequencing on immune cells isolated from lungs of wild type and Ron SF-/- mice 2 or 4 weeks after tumor injection via tail-vein. To study whether sfRon controls T cell function, T cells from wild type or Ron SF-/- mice were cultured ex vivo and T cell activation and exhaustion were examined. Additionally, specific T cell receptors were ectopically expressed on T cells and stimulated with corresponding peptides to access immune modulatory responses. To examine the effector functions, CD8 T cells were stimulated with either specific peptide or co-cultured with antigen-expressing tumor cells and subjected to IFNy detection by flow cytometry or ELISA. To examine the function of helper T cells, peptide-stimulated CD4 T cells were subjected to intracellular detection of IFNy, IL4, IL17A, and IL10, which represent helper effector subsets Th1, Th2, Th17, and iTreg, respectively.

Conclusions Our results showed that expression of sfRon causes defects in T cell recruitment into the metastatic site, and deletion of sfRon maintains T cell homeostasis and prevents T cell anergy caused by overactivation. These data strongly advance the field on the mechanism by which sfRon suppresses T cell responses and sheds light on new therapeutic strategies focusing on Ron kinase inhibitors for the prevention and treatment of metastatic breast cancer.

Ethics Approval All animal procedures were reviewed and approved by the University of Utah Institutional Animal Care and Use Committee; protocol number: 21-06006.