Plasma Autoantibodies Identify Citrullinated Transferrin Receptor Neoepitopes in Small Cell Lung Cancer

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Small cell lung cancer (SCLC) is the 6th leading cause of cancer-related deaths with fewer than 6% of patients surviving 5 years post diagnosis. We have identified 22 autoantibody-antigen complexes upregulated in 3 independent cohorts of SCLC and hypothesized these autoantibodies could elucidate tumor-specific neoepitopes. One of the validated autoantibody-antigen complexes target transferrin receptor (TFRC, CD71), a highly expressed, cell surface protein present in many types of cancer. We found TFRC widely expressed in SCLC tissue microarrays and TFRC transcript was present across SCLC subtypes in 53% of ASCL1+, 27% of NEUROD1+, 71% of POU2F3+, and 33% of YAP1+ cell lines. While TFRC is expressed in rapidly proliferating normal tissues, we have found that TFRC present in SCLC tumors contain post-translationally modified citrulline residues. We also found peptidyl arginine deiminases, the enzymes responsible for converting citrulline from arginine, were highly expressed in SCLC. We identified 5 citrullinated residues in the extracellular domain of TFRC (cit-TFRC) that act as neoantigens targeted by autoantibodies in SCLC patient plasma. Using this information, we isolated cit-TFRC-specific B cells directly from SCLC peripheral blood mononuclear cells via cit-TFRC-peptide tetramers. After single cell sorting and targeted sequencing of the B cells’ antibody variable binding domains, expression in a human IgG backbone allowed us to make antibodies that are specific for cit-TFRC. These human cit-TFRC antibodies recognize cit-TFRC in SCLC cell lines and tumors, but not TFRC expressed in normal human tissues. Using click chemistry the antimitotic drug monomethyl auristatin E (MMAE) was attached to a cit-TFRC antibody to create an antibody drug conjugate with the capability of killing SCLC cell lines *in vitro*. Finally, a far-red fluorescent-labeled cit-TFRC antibody injected by tain vein honed to SCLC sub-cutaneous tumors suggesting the potential for *in vivo* efficacy. Using our unique approach to identify and isolate autoantibodies directly from SCLC patients, we provide early preclinical evidence for the potential to specifically target TFRC in cancer cells- a protein previously thought to be undruggable.