CD47 BLOCKADE MODULATES IMMUNOSUPPRESSIVE CHECKPOINT MOLECULES AND CELLULAR METABOLISM TO SENSITIZE TRIPLE-NEGATIVE BREAST CANCER TUMORS TO IMMUNE CHECKPOINT BLOCKADE THERAPY

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Background Triple-negative breast cancer(TNBC) lacks drugable targets and has high metastatic incidence. Immune checkpoint blockades (ICB) are FDA approved for TNBC treatment, but therapeutic response and biomarkers are limited. CD47 is an integral membrane protein overexpressed on cancer cells that alters anti-tumor immunosurveillance, resulting in tumor progression. CD47 is involved in metabolic reprogramming but whether CD47 is a marker of progression and its role in ICB response for TNBC remains unknown.

Methods Human TNBC biopsies were subjected to immunohistochemical analysis to determine CD47 role in TNBC progression. To determine CD47 impact on tumor burden, a carcinogen-induced TNBC model was performed in female wild type(WT) and cd47 null(cd47^{-/-}) C57Bl/6 mice. To evaluate immune infiltrate signaling, tumors underwent spatial tissue proteomics by multiplexing photo-cleavable antibodies in Formalin-Fixed Paraffin-Embedded samples. An orthotopic EMT-6 murine TNBC model was performed to investigate tumor burden for CD47 monotherapy or in combination with anti-PD-L1 therapy.

Results Human matched primary, and metastatic TNBC biopsies increased immunoreactivity to CD47, signifying a potential therapeutic target(n=24). CD47 deficiency in the carcinogen-induced DMBA model decreased tumor incidence, weight, and area compared to WT(n=8/group,*p<0.003). Since CD47 can regulate metabolism, tumors underwent metabolomic analysis. Principal component analysis displayed differentially regulated metabolites between WT and cd47^{-/-} tumors. Decreased carnitine conjugated fatty acids and ketone bodies were observed in cd47^{-/-} tumors compared to WT, suggesting decreased fatty acid availability and/or metabolism(n=9/group,*p<0.05). TNBC cell respiratory measurements validated that targeting CD47 shifted metabolic dependency from fatty acid oxidation to glycolysis(n=3,*p<0.05). Kynurenine/tryptophan pathway metabolites, which catabolize Indoleamine-2,3-dioxygenase (IDO1) and involved in anti-PD-1/PD-L1 resistance, were decreased in cd47^{-/-} tumors compared to WT (n=9/group,*p<0.05). Spatial proteomic analysis determined that cd47^{-/-} tumors had elevated immune cell infiltration (CD45+, CD3+), suggesting CD47 absence enhances tumor immunogenicity and immune-mediated tumor ablation. Multiplexing of photo-cleavable antibodies increased protein expression of immune checkpoint molecules (PD-L1,VISTA,B7-H3,BatF3) and immunosuppressive cell types (CD11b+,Ly6c+) in WT tumors compared to cd47^{-/-}, suggesting CD47 absence limits immunosuppressive signaling (n=16/group,*p<0.05). Since anti-PD-L1 therapies are approved to treat TNBC and WT tumors have PD-L1 upregulation, we examined how targeting CD47 would impact tumor burden of mice receiving anti-PD-L1 therapy. Targeting CD47 or PD-L1 as monotherapy decreased tumor burden; however, in combination it further reduced tumor burden compared to anti-PD-L1 treatment due to increased intratumoral granzyme B secreting cytotoxic T cells(n=4-8/group,*p<0.05).

Conclusions Our data indicates that CD47 may serve as a marker of anti-PD-L1 response, and targeting CD47 enhances immunogenicity and decreases immunosuppressive molecules, sensitizing TNBC tumors to anti-PD-L1 therapy to reduce tumor burden.

Acknowledgements DSP is supported by the NCI R21 (CA249349) and the American Cancer Society Research Scholar Grant (133727-RSG-19-150-01-LIB). ERS is supported by the NIAID Immunology and Pathogenesis T32 Training Grant (T32AI007401).

Ethics Approval Animal studies were approved by the Institutional Care and Use Committee, Wake Forest Health Sciences.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.616>