

## Targeting SIRP $\alpha$ as a therapeutic strategy for the treatment of breast cancer brain metastasis

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Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer characterized by the lack of specific targets and an incidence of brain metastasis from the primary site of approximately 35%. There is no standard treatment for managing brain metastasis associated with TNBC; therefore, new strategies are urgently needed to overcome disease mortality. The CD47/SIRP $\alpha$  signaling pathway is implicated in tumor progression due to bypassing innate and adaptive immune surveillance. Most strategies targeting this pathway focus on targeting the receptor CD47; however, targeting SIRP $\alpha$  as a potential strategy to mitigate tumor burden remains understudied. Analysis of gene expression database shows that SIRP $\alpha$  expression is significantly elevated in invasive breast cancer compared to primary. Furthermore, single-cell data indicates that SIRP $\alpha$  is expressed in basal epithelial cells in TNBC tumors, aside from the myeloid compartment. Our immune staining against SIRP $\alpha$  in breast cancer patient biopsies shows a 3.5-fold increase in SIRP $\alpha$  expression in metastatic lesions compared to the primary tumor (n=19; p  $\leq$  0.01). To confirm that SIRP $\alpha$  is expressed on triple-negative cancer cells and whether it may be increased in brain metastatic cells, we stained 4T1 parental and brain-trophic 4T1-Br3 cells and found an 84% increase in SIRP $\alpha$  in the metastatic cells (n=3; p  $\leq$  0.05). Furthermore, Agilent xCELLigence Real-Time Cell Analysis revealed that SIRP $\alpha$  blockade inhibits brain-trophic 4T1br3 cell migration (n=4; p $\leq$ 0.01). Therefore, targeting SIRP $\alpha$  may be a new immunotherapeutic strategy to treat TNBC brain metastasis. Anti-SIRP $\alpha$  treatment of mice bearing brain-trophic 4T1br3 orthotopic tumors showed reduced tumor volume and tumor weight by over 50% compared to isotype control-treated mice (n=6; p  $\leq$  0.05). Furthermore, in a model of intracardial brain metastasis, treatment with SIRP $\alpha$  antibody was associated with a 40% increase in survival on day 15 compared to isotype control-treated mice. SIRP $\alpha$  blockade also reduced metastatic brain lesion formation by approximately 90%, determined by IVIS imaging (n=4-7; p  $\leq$  0.05). Nanostring GeoMX digital spatial profiling of the brain lesions revealed the immune checkpoints cluster of differentiation 152 (CTLA4), programmed cell death protein 1 (PD-1), programmed death ligand-1 (PD-L1), and cluster of differentiation 276 (CD276 or B7-H3) were significantly reduced in SIRP $\alpha$  treated brain lesions (n=3-6; p  $\leq$  0.05). Additionally, the extracellular matrix protein fibronectin, which contributes to invasion, metastasis, and immune evasion, was reduced by 70% in SIRP $\alpha$  treated brain lesions (n=3-6; p  $\leq$  0.05). These data suggest that SIRP $\alpha$  blockade may influence tumor and innate immune cells to limit brain metastatic breast cancer growth and enhance survival.