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Minocycline Enhanced T-cell Cytotoxicity of Tumor Cells

Cancer cells propagate by finding means to hide from the immune system. Immunotherapies allow the immune system to recognize and attack the hidden cancer cells reducing the spread of the disease. Immune checkpoint inhibitors are widely used for immunotherapies as they identify and block proteins that cancer cells use to hide from the immune system. Some common cancer immunotherapies such as immune checkpoint inhibitors (ICIs) and anti-programmed cell death-1 (PD-1) therapy have failed in many patients due to target receptor expression irregularities.[1] Therefore, the development of novel immunotherapies based on different mechanisms is much needed.

In the past, attempts have been made to develop cancer immunotherapy agents based on a drug-repurposing approach. For example, drugs like Metformin and Bezafibrate have been used to increase antitumor immunity apart from their primary functionality. [2&3] Interestingly, Tetracyclines have been reported to enhance T-cell immunity in vitro using a bispecific T-cell engager (BITE) which was specific for CD3 expressed on T cells and an antigen expressed on tumor cells. [4] However, their underlying mechanism of action needs to be clarified for the development of novel cancer immunotherapy.

In this study published in the Journal for Immunotherapy of Cancer, researchers from Osaka University have discovered that tetracyclines attack the immunosuppressive proteins produced by cancer cells and thereby helping the immune system to find the cancer cells[5] as shown in **Figure 1**.

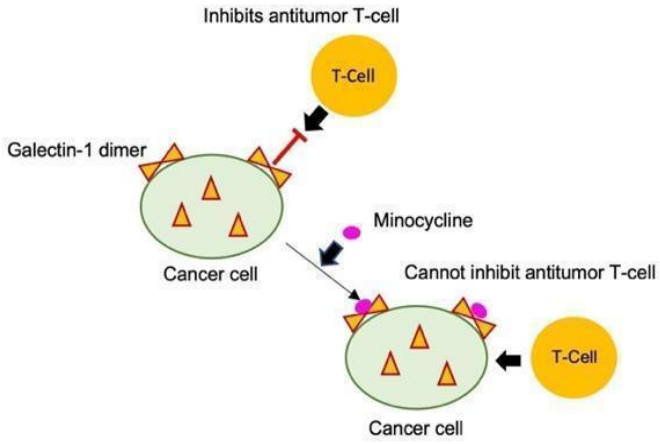

Figure 1: Schematic of Minocycline binding to Galectin-1 dimer increasing antitumor immunity.

Galectin-1 is one such immunosuppressive protein found predominantly in cancer cells which induces apoptosis of T cells before they destroy tumor cells.[6] Studies have shown that Galectin-1 is a highly unstable dimer when isolated for biophysical characterization and kinetic studies. Additionally, the conformation of the extracted Galectin-1 might be different than that of the same protein in the membrane milieu. Also, the extraction can alter the natural function and binding behaviour of the receptor. Therefore, measurements of the binding affinity and kinetic parameters of membrane proteins in their natural environment using Surface plasmon resonance (SPR) microscopy is the most accurate and biologically relevant option to study molecular interactions at the cell surface level.

SPRm 200, which integrates label-free, high-resolution SPR microscopy's kinetic measurements with bright-field optical imaging was used to study the binding between Minocycline and Galectin-1 found on U251 cancer cells. Wild-type U251 cells were transfected with a Galectin-1 CRISPR/Cas9 KO plasmid or control CRISPR/Cas9 plasmid to generate two strains (Galectin-1 (LGALS1 gene) knockout U251 cells and control/wild-type U251 cells.

Increased expression of Galectin -1 in the wild type compared to the knockout strain was confirmed using ELISA. The SPRm 200 chips were coated with poly-D-lysine before 3×10^4 galectin-1 knockout or wild-type U251 cells were plated. After a 24-hour culture at 37°C with 5% CO_2, cells were fixed for 10min with 4% formaldehyde. Minocycline binding to galectin-1 knockout or wild-type U251 cells was measured using the SPRm 200AP system.

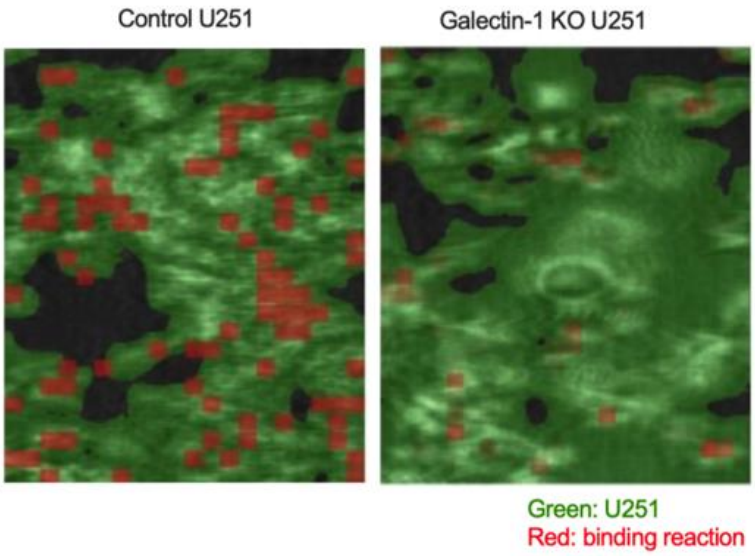

Figure 2: SPRm 200 results of binding of Minocycline to Galectin-1. The green site shows the presence of tumor cells (control U251 and Galectin-1 KO U251) on measurement chips and the red site shows the binding interaction of minocycline to tumor cells. [5]

SPRm 200 cell-binding experiments showed increased binding of Minocycline to the surface of Galectin-1-expressing U251 cells compared to that of Galectin-1 knockout U251 cells, represented by red squares (**Figure 2**). Galectin-1 is reportedly a potent suppressor of not only antitumor T-cell activity, but also antitumor NK immune surveillance.[7] Blocking the effects of Galectin-1 on T-cells using drugs like minocycline might just be the key to new cancer treatments.

Tetracyclines have a different mechanism of action from the commonly used immune checkpoint inhibitors and immunotherapies used to treat cancer. Understanding this new approach using SPRM can lead to the development of new drugs that target the immune system and benefit cancer patients who don't benefit from the current therapies.

References:

1) Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015;372:2018–28.

2) Eikawa S, Nishida M, Mizukami S, et al. Immune-mediated antitumor effect by type 2 diabetes drug, metformin. Proc Natl Acad Sci U S A 2015;112:1809–14.

3) Nishida M, Yamashita N, Ogawa T, et al. Mitochondrial reactive oxygen species trigger metformin-dependent antitumor immunity via activation of Nrf2/Mtorc1/P62 axis in tumor-infiltrating Cd8t lymphocytes. J Immunother Cancer 2021;9:e002954.

4) Noguchi Y, Yamamoto Y, Iwahori K, et al. Tetracyclines enhance antitumor T-cell responses induced by a Bispecific T-cell Engager. Biol Pharm Bull 2022;45:429–37.

5) Tone M, Iwahori K, Hirata M, et al. Tetracyclines enhance antitumor T-cell immunity via the Zap70 signaling pathway. J Immunother Cancer 2024;12:e008334.

6) Cedeno-Laurent F, Dimitroff CJ et al. Galectin-1 research in T cell immunity: past, present and future. Clin Immunol 2012;142:107–16.

7) Baker GJ, Chockley P, Yadav VN, et al. Natural killer cells eradicate Galectin-1-deficient glioma in the absence of adaptive immunity. Cancer Res 2014;74:5079–90.