Cancer-cell stiffening via cholesterol depletion enhances adoptive T-cell immunotherapy

Li Tang[1,2*] Kewen Lei[1], Armand Kurum[1],
[1]Institute of Materials Science & Engineering, [2]Institute of Bioengineering, École polytechnique fédérale de Lausanne (EPFL), Lausanne, Switzerland, CH-1015.*corresponding author: li.tang@epfl.ch

Statement of Purpose: Tumours employ certain immune inhibitory pathways, termed immune checkpoints, to evade anti-tumour immunity, in particular, T-cell mediated cytotoxicity. Blockade of the ligand-receptor based immune checkpoints using antibodies, such as anti-cytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) and anti-programmed cell death protein 1 (anti-PD-1) antibodies, can reactivate anti-tumour immunity and has led to remarkable clinical success in cancer immunotherapy. Identifying new checkpoints and blocking them with therapeutic interventions could potentially benefit patients broadly, especially those who fail to respond to current checkpoint blockade immunotherapies. Despite substantial efforts in seeking new checkpoints based on biochemical signals, potential inhibitory pathways involving biomechanical signals, such as target cell stiffness, remain largely unexplored. Although tumours are typically stiffer than the paired normal tissues due to the aberrant production and crosslinking of extracellular matrix proteins, individual cancer cells are generally softer than their non-malignant counterparts. Cellular softness is a biomechanical characteristic, which is correlated with the transformation, malignancy, and metastasis of cancer cells. The decrease in cancer cell stiffness has been shown to arise from softening of both cytoskeletal network and plasma membrane. T-cells directly interact with the surface of target cells; thus, the mechanical properties of cell cortical structures, including the plasma membrane and the underlying actin cortex, may impact cancer cell–T-cell interactions. Nevertheless, the role of cancer cell stiffness in evading immunosurveillance remains elusive.

Methods: Cortical stiffness of cancer cells was augmented by depleting cholesterol in the membrane lipid bilayer using methyl-β-cyclodextrin (MeβCD). Overcoming this mechanical immune checkpoint (target cell softness/T-cell mechano-sensing axis) by stiffening cancer cells markedly improves the anti-tumour efficacy of adoptive T-cell transfer (ACT) immunotherapy against solid tumours. We further provide evidence that the enhanced cytotoxicity against stiffened cancer cells is mediated by increased T-cell forces but not known cytotoxic pathways based on biochemical signals.

Results and Discussion: Malignancy and tumour progression are associated with cancer-cell softening. Yet how the biomechanics of cancer cells affects T-cell mediated cytotoxicity and thus the outcomes of adoptive T-cell immunotherapies is unknown. T-cells do not only sense mechanical environments but also exert forces at the immunological synapse to potentiate cytotoxicity against target cells. Cytoskeletal forces and effector cytokine production are substantially reduced when T-cells sense a soft substrate surface or soft target cells. Inspired by these observations, we hypothesized that cancer cells utilized cellular softness as a mechanical immune checkpoint to develop resistance toward T-cell mediated cytotoxicity by impairing T-cell mechanical forces. Here, we show that T-cell-mediated cancer-cell killing is hampered for cortically soft cancer cells, whose plasma membrane is enriched with cholesterol, and that cancer-cell stiffening via cholesterol depletion augments T-cell cytotoxicity and enhances the efficacy of adoptive T-cell therapy against solid tumours in mice (**Figure 1**). We also show that the enhanced cytotoxicity against stiffened cancer cells is mediated by augmented T-cell forces arising from an increased accumulation of filamentous actin at the immunological synapse, and that cancer-cell stiffening has a negligible influence on T-cell receptor signalling, on the production of cytolytic proteins such as granzyme B, on the secretion of interferon gamma and tumour necrosis factor alpha, and on Fas-receptor–Fas-ligand interactions. Our findings reveal a mechanical immune checkpoint that could be targeted therapeutically to improve the effectiveness of cancer immunotherapies.

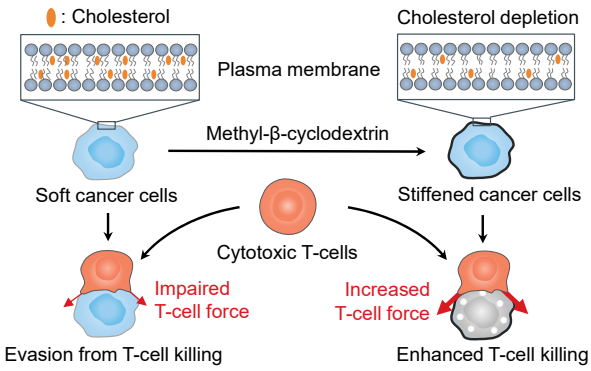

Figure 1. Schematic illustration of mechanical immuno-suppression induced by the softness of cancer cells, which could be overcome by stiffening cancer cells for enhanced cancer-cell killing mediated by T-cells.

References:

Lei, K.; Kurum, A.; Kaynak, M.; Bonati, L.; Han, Y.; Cencen, V.; Gao, M.; Xie, Y.-Q.; Guo, Y.; Hannebelle, M.T.M.; Wu, Y.; Zhou, G,; Guo, M.; Fantner, G.E.; Sakar, M.S.; Tang, L.* "Cancer-Cell Stiffening via Cholesterol Depletion Enhances Adoptive T-cell Therapy", ***Nat. Biomed. Eng.*** **2021**, *in press*.