1. Abstract (EN)

The adoptive cellular immunotherapy (ACI) based on *ex vivo* produced T cells is a modern treatment modality of cancer. However, the *ex vivo* production of T cells with high therapeutic efficacy is far to be well established. Wnt/β-catenin and mTOR signaling have been shown to affect both cancer cells and immune cells. Therefore, the modulation of these pathways seems to be perspective for the production of T cells with superior therapeutic efficacy. The aim of our project was to investigate, how interventions into Wnt/β-catenin and mTOR signaling during the *ex vivo* production of tumor-associated antigen-specific T cells could improve the production of T cells with a desired and controlled phenotype that would best fit for use in ACI of cancer.

In the first part of our study, we investigated the role of Wnt/β-catenin inhibition by XAV939 on cancer cell elimination by lymphocytes from patients with localized biochemically recurrent prostate cancer (BRPCa). We found that preconditioning BRPCa lymphocytes with 5 μM XAV939 accelerated the elimination of LNCaP and PC3 cells during the coculturing. However, during subsequent re-coculturing with fresh LNCaP cells, BRPCa lymphocytes were no longer able to eliminate cancer cells unless coculturing and re-coculturing were performed in the presence of the 5 μM XAV929. These data indicate that modulation of Wnt/β-catenin signaling pathway increases the "immunosensitivity" of cancer cells and licenses lymphocytes from prostate cancer (PCa) patients to tumor cell elimination.

In the second part of our study, we attempted to determine if modulation of Wnt/β-catenin and mTOR signaling pathways could affect the function of antigen-experienced and expanded $CD8^+$ T cells. For the study, we used 3 inhibitors: TWS119, an inhibitor of GSK-3β, which is the upstream regulator of mTORC1 and Wnt/β-catenin signaling; rapamycin, an inhibitor of mTORC1 signaling; Torin 1, an inhibitor of both arms of mTOR signaling, mTORC1 and mTORC2. We found that acute preconditioning of antigen-experienced and expanded $CD8^+$ T cells with TWS119 and rapamycin led to the re-expansion of $CD8^+$ T cells that showed increased distal inflammatory response. On the other hand, no such effect was observed with Torin 1. These data indicates that the GSK-3β-mTORC1 axis is the important regulator of effector functions in antigen-experienced CD8+ T cells.

In summary, although Wnt/β-catenin and mTOR signaling pathways in cancer cells have become an attractive target for the treatment of multiple cancers, its dual impact on immune cells and other cells makes it's *in vivo* targeting for therapeutic purposes controversial. However, it's *ex vivo* modulation in selected immune cells under controlled conditions may, on the other hand, bring a technological advantage in the production of cells for ACI of cancer.