Abstract

Chimeric antigen receptors (CARs) are artificial molecules composed of an antibody derived antigen recognition domain which is fused with the signal transduction domain derived from the physiological TCR. CAR technology used to transduce patients T-cells and endow them with the specificity to a certain surface antigen, has been a major breakthrough in cancer immunotherapy in the last decade. This strategy has been most successful for treating hematologic malignancies. Various CAR approaches and applications are currently tested mainly in the United States where many clinical trials have been launched. In contrast, in the Czech Republic, there are only a few teams focused on this topic with no clinical trials going on.

During my work on this diploma thesis and in close collaboration with MUDr. Pavel Otáhal, PhD., who is working on implementation of CAR technology into the Czech clinics for the treatment of B-cell malignancies, individual functional CARs were prepared and tested. CAR expressing Jurkat T-cell lines were generated using a lentiviral vector transduction system. CAR functionality was determined by two different assays. We have shown that individual CARs are able to recognize the B-cell lineage specific antigens CD19 and CD20 and significantly up-regulate the activation molecule CD69 upon T-cell activation by co-cultivation with RAJI B cell used as a target. Individual CAR constructs also showed to be functional in the mouse thymoma cell line with NFAT-GFP reporter. Our ultimate goal, the preparation of a superCAR construct that would endow T-cell with dual specificity against both CD19 and CD20 antigens has not been accomplished yet and the work on this construct is still in the process.

Within the work frame we have prepared suitable conditions for further experimental testing of CAR technology *in vitro*. Future perspective of this work relates to the completion of the superCAR construct. We hopeful that that newly designed dual specificity CAR construction would prove efficient in preventing malignant B-cells that have lost the expression of one of the B-cell lineage specific antigens, in order to escape their detection. The CAR technology for cancer immunotherapy is a perspective therapeutic strategy worth of research in the Czech.

Key words: treatment of leukemia, T cells, TCR, chimeric antigen receptor, B cells, CD19, CD20, co-stimulation, immunotherapy