



UNIVERSITÀ DEGLI STUDI DI TORINO

DEPARTMENT OF MEDICAL SCIENCES

Master's Degree in Medical Biotechnology

MASTER THESIS

Class LM-9

**Diacylglycerol kinase alpha promotes ubiquitination
and cell surface downregulation of chimeric antigen
receptor**

Supervisor:

Prof. Andrea Graziani

Candidate:

Alessia Labate

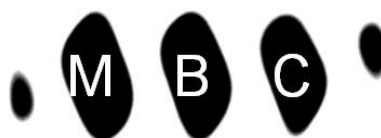
Co-supervisor:

Dr. Valeria Malacarne

Student ID:

1034012

Università di Torino



Molecular Biotechnology Center

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Abstract

Loss of chimeric antigen receptor (CAR) surface expression emerged as a key determinant in CAR-T cell dysfunction. However, the underlying mechanisms directing CAR trafficking remain largely unknown. Current strategies to enhance the anti-tumour effectiveness of CAR-T cells involve targeting of negative regulators of T cell signalling, such as diacylglycerol kinase α (DGK α), which inhibit diacylglycerol (DAG)-driven TCR signalling by converting DAG to phosphatidic acid (PA). Although it is widely accepted that its kinase activity does play a role in limiting CAR-T cell function, several evidence suggest that it may act more upstream in the TCR signalling. Here, we showed that in DGK α depleted CAR-T cells, the engagement of tumour antigen result in a reduced loss of CARs from the cell surface compared to WT CAR-T cells. Reconstitution with both DGK α WT or kinase dead (KD) mutant reduced the amount of the receptor at the cell surface, suggesting a new kinase-independent function of DGK α in CAR regulation. Once internalized, CAR is saved from antigen-induced lysosomal degradation in DGK α KO CAR-T cells, due to a reduction of the E3 ubiquitin ligase Cbl recruitment to the receptor, and rather recycled back to the plasma membrane. Thus, DGK α is responsible for CAR ubiquitination, degradation and finally for the reduction of the CAR at the cell surface upon antigen stimulation. Together, these data indicate that DGK α couples antigen-induced T cell activation to the triggering of the negative feedback mechanism that lead to lysosomal degradation of the activated CAR.