CTLA-4 (CD152) impairs cytotoxic T-lymphocyte responses via PDCD4 induction

H. Lingel[1], J. Wissing[2], F. Klawonn[2], A. Arra[1], M. Pierau[1], L. Jänsch[2], M. Brunner-Weinzierl[1]

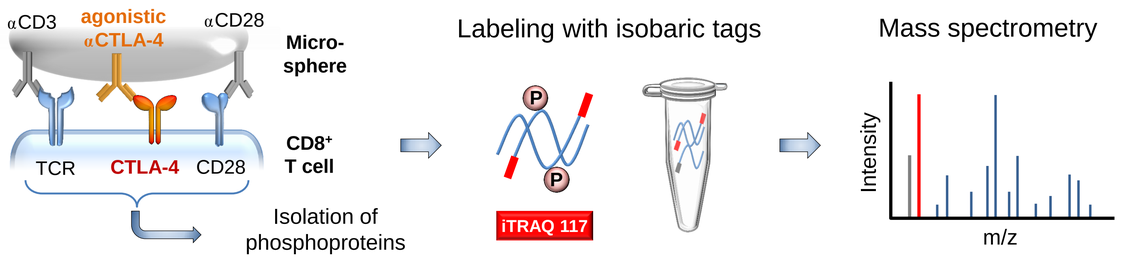
1 Otto-von-Guericke University, Magdeburg, Germany;

2 Helmholtz Centre for Infection Research, Braunschweig, Germany.

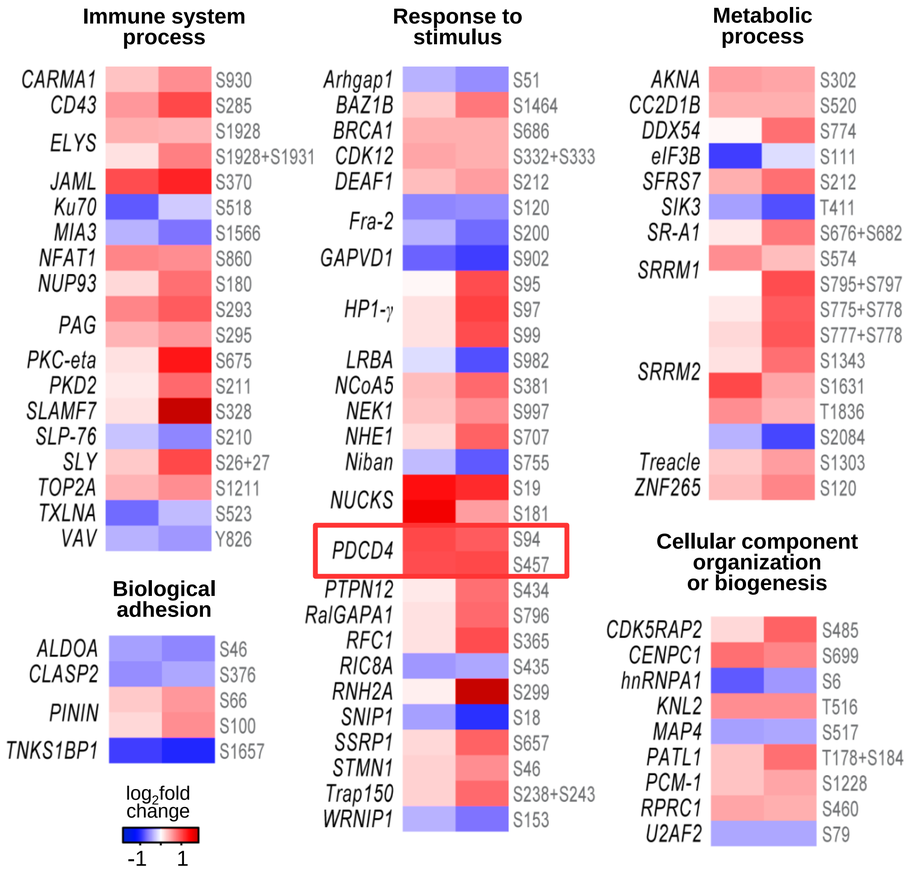
ABSTRACT

Inhibitory T-cell surface receptors like Cytotoxic T-lymphocyte-associated Protein-4 (CTLA-4) and Programmed cell death 1 (PD-1) play a crucial role in the termination of adaptive immune responses and promote the functionally impaired state of CD8⁺ T cell exhaustion. Their blockade is being used in immune-checkpoint therapy as a promising approach to restore effective T-cell responses against tumors. However, the intracellular pathways triggered by these receptors still remain incompletely understood. To determine target molecules downstream of CTLA-4, an accurate mass spectrometry analysis was performed. The dataset revealed that the engagement of CTLA-4 led to altered phosphorylation of proteins involved in T-cell signaling, DNA replication, RNA processing and microtubule polymerization. Beside other targets, a CTLA-4-induced expression of the translational inhibitor Programmed cell death 4 (PDCD4) could be revealed and characterized. This mechanism was responsible for the restriction of cytotoxic T-lymphocyte effector functions. Accordingly, the deficiency of PDCD4 led to superior control of melanoma growth *in vivo*. These findings point out that targeting of PDCD4 could provide a potent strategy to improve anti-tumor immunotherapy.

METHODS

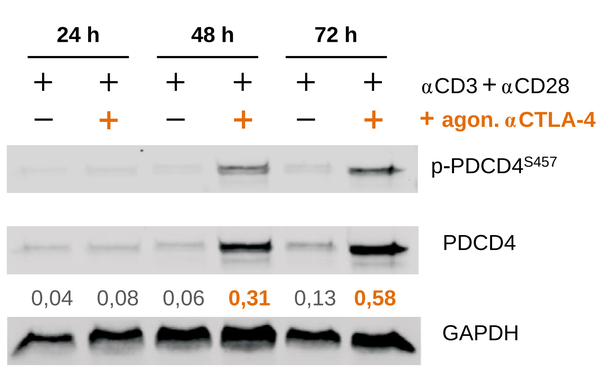


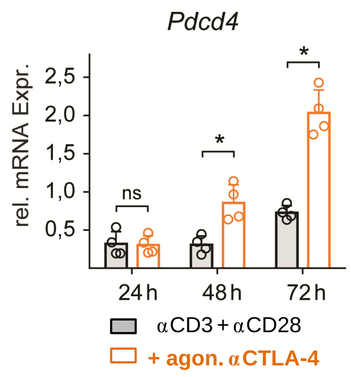
iTRAQ mass spectrometry enabels simultaneous analysis of phosphoproteins.
Phosphorylated proteins were isolated from CD8⁺ T cells differentiated with anti-CD3/CD28 and additional CTLA-4 engagement or not. Comparative analysis of phosphoprtein abundance was performed using isobaric tags and LC-MS.



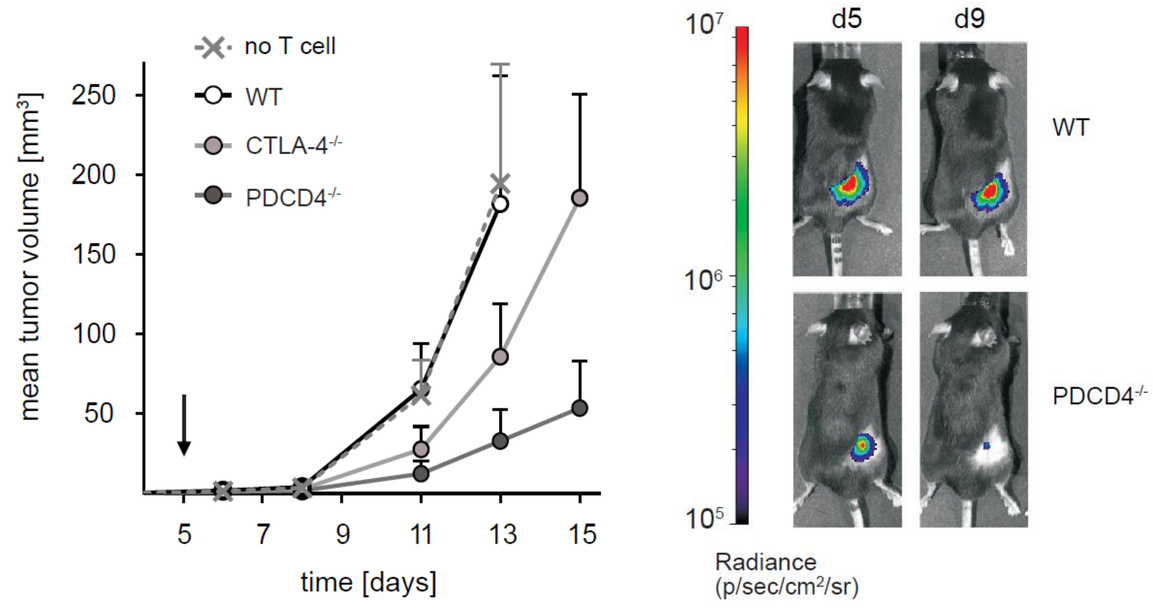
CTLA-4 modulates the phosphoproteome in differentiating CD8⁺ T cells.
Phosphorylation profile of significantly regulated proteins in CD8⁺ T cells 48 h after differentiation with anti-CD3/CD28 and additional CTLA-4 engagement, acquired by iTRAQ mass spectrometry. Proteins were functionally clustered. Blue and red represent low and high relative phosphorylation, respectively.

RESULTS

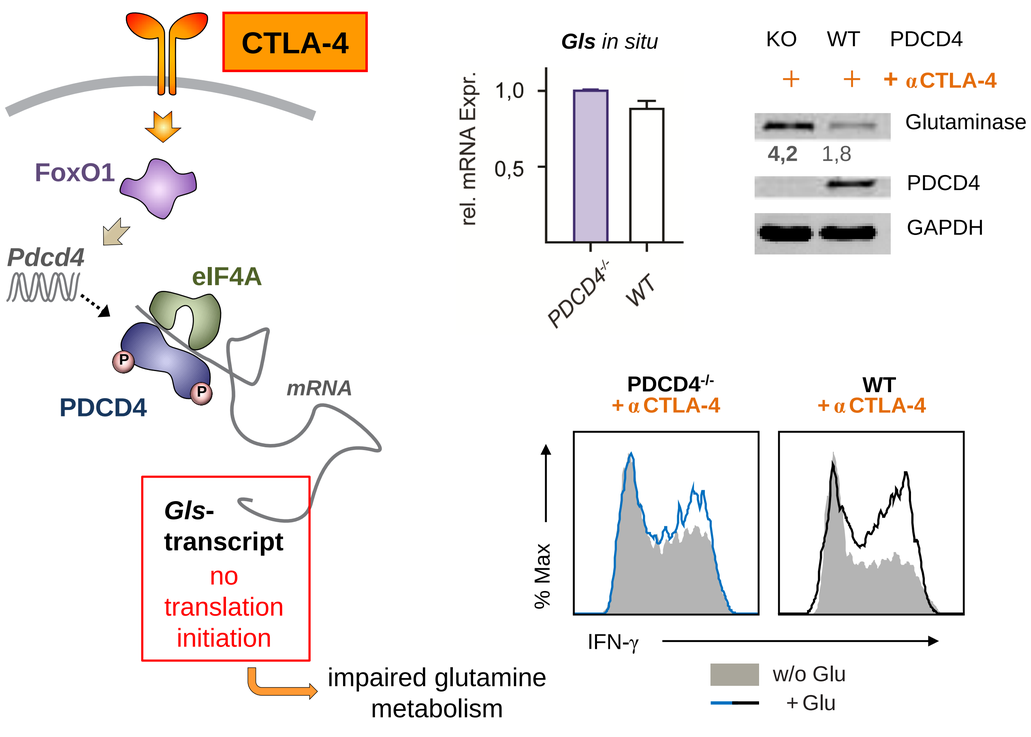




CTLA-4 induces PDCD4 expression in differentiating CD8⁺ T cells.
(left) Immunoblot analysis of phosphorylated and total PDCD4 in CD8+ T cells from 24 h to 72 h. (right) *Pdcd4* mRNA expression profile after differentiation with or without additional agonistic αCTLA-4.



PDCD4 deficiency leads to enhanced control of experimental tumors.
(left) Tumor volume of mice s.c. inoculated with OVA-expressing B16 melanoma, followed 5 days later by i.v. transfer of no T cells (crosses) or naïve TCR-transgenic CTLA-4 and PDCD4 wild-type (WT, white shaded) or CTLA-4- (light gray shaded) or PDCD4-deficient (dark gray shaded) OT-I CD8+ T cells. (right) Bioluminescence images of PDCD4 WT and deficient mice from day 5 or day 9 after subcutaneous transplantation of luciferase-expressing TRAMP-C1 prostate cancer cells.



CTLA-4 impairs CD8⁺ T-cell glutamine metabolism and effector functions.
(left) CTLA-4 re-activates FoxO1 leading to PDCD4 expression. PDCD4 binds *Gls* mRNA inhibiting glutaminase protein translation. (right) Glutaminase expression (upper panel) and IFN-γ production in the presence or absence of exogenous glutamate (lower panel) in OT-I PDCD4$^{-/-}$ or OT-I PDCD4$^{+/+}$ (WT) CD8⁺ T cells after activation with additional agonistic αCTLA-4.

CONCLUSIONS

- CTLA-4 engagement induces defined post-transcriptional and translational changes in differentiating CD8⁺ T cells.
- CTLA-4-induced PDCD4 is a novel pathway that inhibits CD8⁺ T-cell functions by controlling metabolic demands
- Selective targeting of inhibitory molecules like PDCD4 leads to enhanced anti-tumor immune responses