Dissecting the pro-tumoural role of the essential amino-acid transporter complex CD98/LAT1

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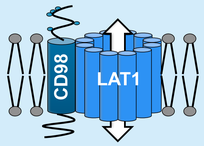
Introduction

The **CD98/LAT1** heterodimer is a **multifunctional** transmembrane complex that **is overexpressed in many cancers** and is a **bad prognostic** marker

The **CD98** glycoprotein interacts with **integrins** to regulate **migration, proliferation** and **adhesion**-induced intracellular signaling.

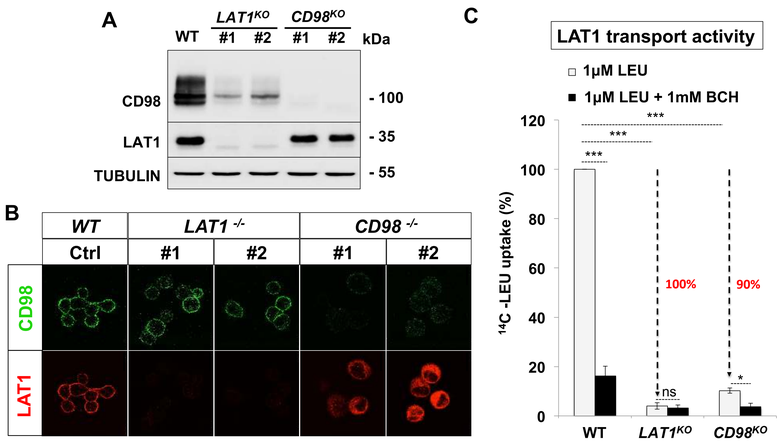


LAT1 mediates the **transport** of **essential** large neutral **amino acids** in mammals and is able to activate the **mTORC1** pathway.

In this study, we assessed the pro-tumoural role of each component of the CD98/LAT1 complex using genetic knock outs of the colorectal adenocarcinoma cell line LS174T

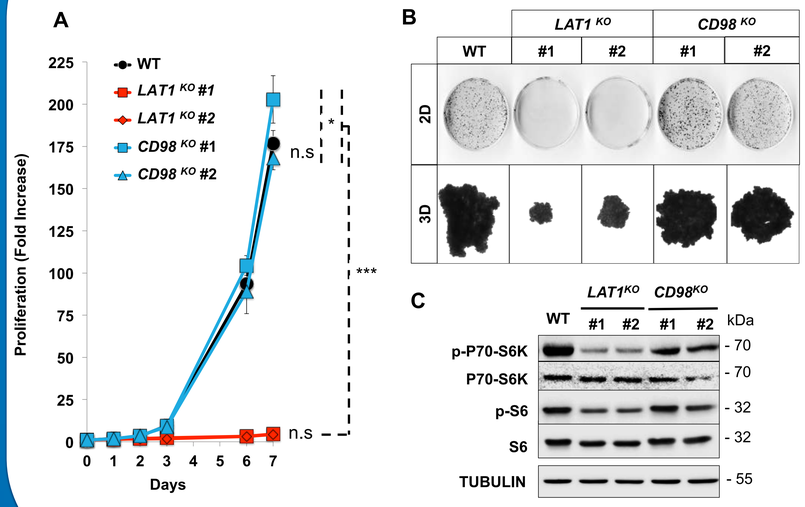
Results

FIG.1: CD98 and LAT1 expression, localization and activity are interdependent



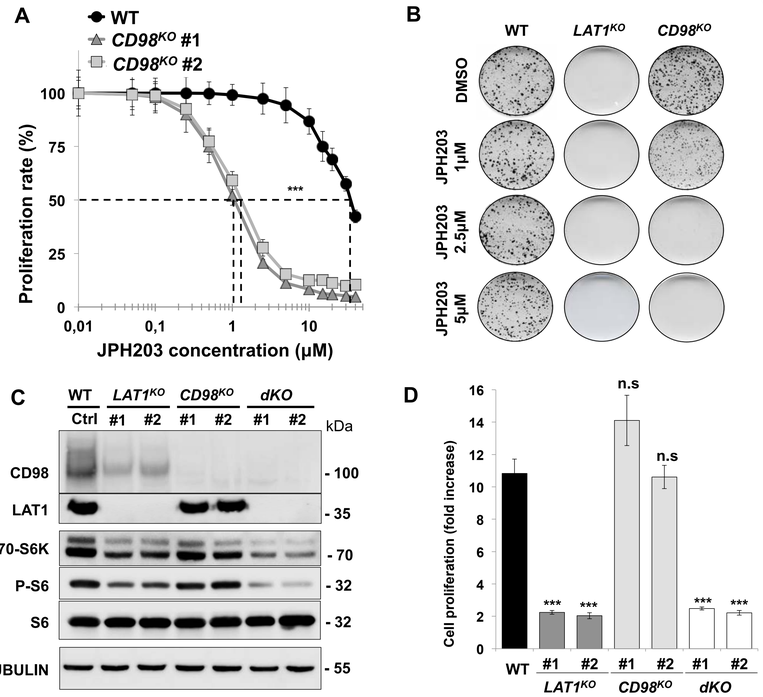
(A) Western blot. **(B)** Confocal. **(C)** ^{14}C Leucine (LEU) transport assay with or without the LAT inhibitor BCH

FIG.2: LAT1 knockout strongly decreases mTORC1 activity and cancer cell proliferation while CD98 invalidation has no effect



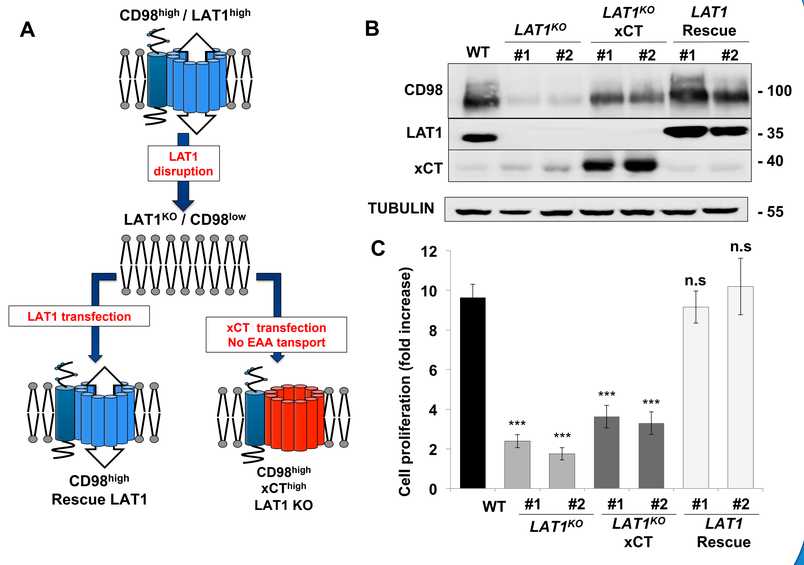
(A) Proliferation Assay **(B)** Clonogenicity (2D) and Spheroid Assay (3D)
(D) Western blot analysis of the mTORC1 activity.

FIG.3: Genetic disruption or pharmacological inhibition of LAT1 abolishes mTORC1 and growth of CD98KO cells



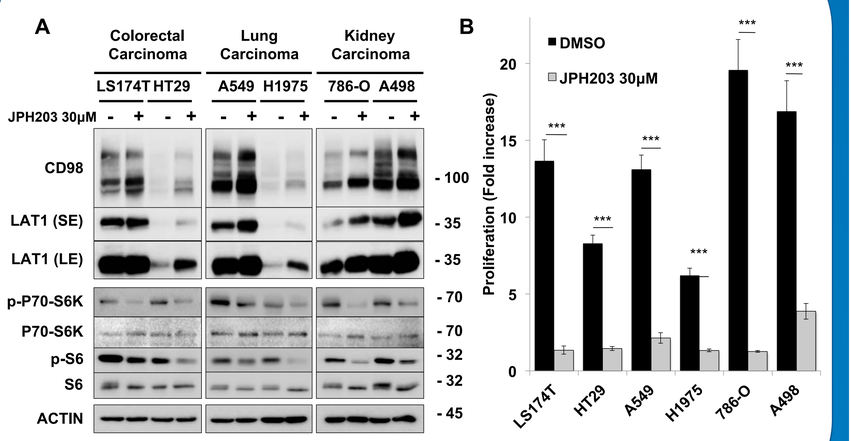
(A) Dose response analysis of the LAT1 specific inhibitor JPH203 in LS174T WT and CD98KO cells
(B) Clonogenicity assay using different concentrations of JPH203 **(C)** Western blot analysis of the CD98, LAT1 expressions and mTORC1 activity in WT, LAT1KO, CD98KO and double KO (dKO : CD98 & LAT1 KO) cells.
(D) Proliferation assay

FIG.4: Rescued CD98 expression in LAT1KO cells does not restore proliferation



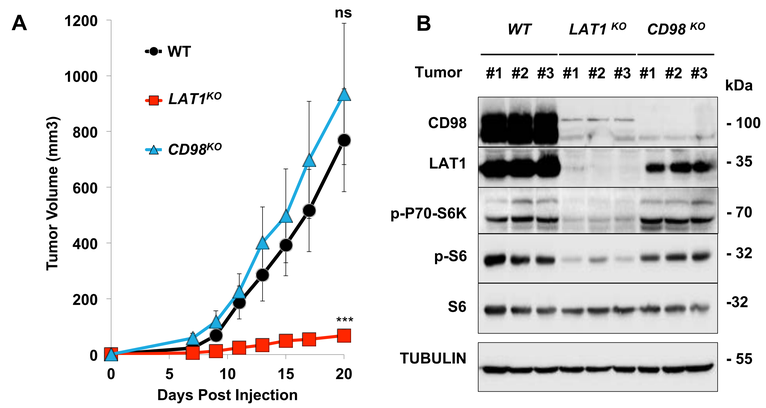
(A) Experimental strategy **(B)** Western blot analysis of CD98, LAT1 and xCT expressions
(C) Proliferation assay

FIG.5: Pharmacological inhibition of LAT1 suppresses mTORC1 activity and proliferation in multiple cancer cell lines



(A) Western Blot analysis of of the CD98, LAT1 expressions and mTORC1 activity in 6 independants cancer cell lines -/+ JPH203 (30µM) **(B)** Proliferation assay of 6 independent cell lines -/+ JPH203 (30µM)
These experiments has been performed using the commercial F12 media containing physiological essential amino acid concentration

FIG.6: LAT1 is essential for mTORC1 activity and tumour growth in vivo while CD98 is dispensable



(A) Xenograft **(B)** Western blot of tumor extracts

Conclusion

- **LAT1** is **essential** for **mTORC1 activity** and **tumour growth**
- These roles of **LAT1** are **independent** of the **CD98 / integrin axis**
- **Targeting LAT1** activity is a **promising therapeutic strategy** in **multiple cancer types**
- Tumour cells tested appear to **lack a redundant mechanism** for essential amino acids uptake **strengthening the idea of developing the LAT1 inhibitor JPH203 as an anticancer strategy in the clinic**

(See Cormerais et al. Cancer Research 2016 June 14, In Press)