GRANT 006_FC_2015

Ruolo patogenetico della chinasi mTOR nella malattia celiaca

TITOLARE DEL GRANT: Prof. Giovanni Monteleone

ISTITUTO: Università Tor Vergata di Roma

DURATA DEL PROGETTO: 3 anni

GRANT 006_FC_2015

Background

- Celiac disease (CD) is an enteropathy triggered by the ingestion of gluten proteins in genetically predisposed individuals (haplotypes DQ2/DQ8+)

- The ingestion of gluten triggers both **innate and adaptive immune responses**, with the production of huge amounts of cytokines, which are supposed to play a key role in the pathological process leading to the mucosal injury

- Phenotypic analysis of the effector T cells infiltrating the duodenum of CD patients has revealed that the disease is marked by activation of gluten-specific CD4+ T cells, which are polarized along the **Th1 pathway**, as they produce mostly interferon (IFN)-γ

- CD-related inflammation is also characterized by the production of the Th17-related cytokine, **IL-17A**, even though this seems to occur through a gluten independent mechanism

Monteleone et al. J Immunol 2010
Meresse et al. Immunity 2012

Background

- In CD mucosa, there is excessive production of **IL-21**, a cytokine made by gluten-specific activated CD4+ T cells and involved in the activation of multiple inflammatory signals in the gut
- IFN-γ-secreting T cells rather than IL-17A-secreting T cells synthesize IL-21, thus suggesting that Th1 cells are the major sources of this cytokine in CD
- **IL-15** stimulates CD4+ T cells to make IL-21 and inhibition of **Akt**, which is up-regulated in active CD, by wortmannin reduced IL-15-driven CD4+ T cell-derived IL-21

Fina et al. Gut 2008
Sarra et al. Mucosal Immunol. 2013

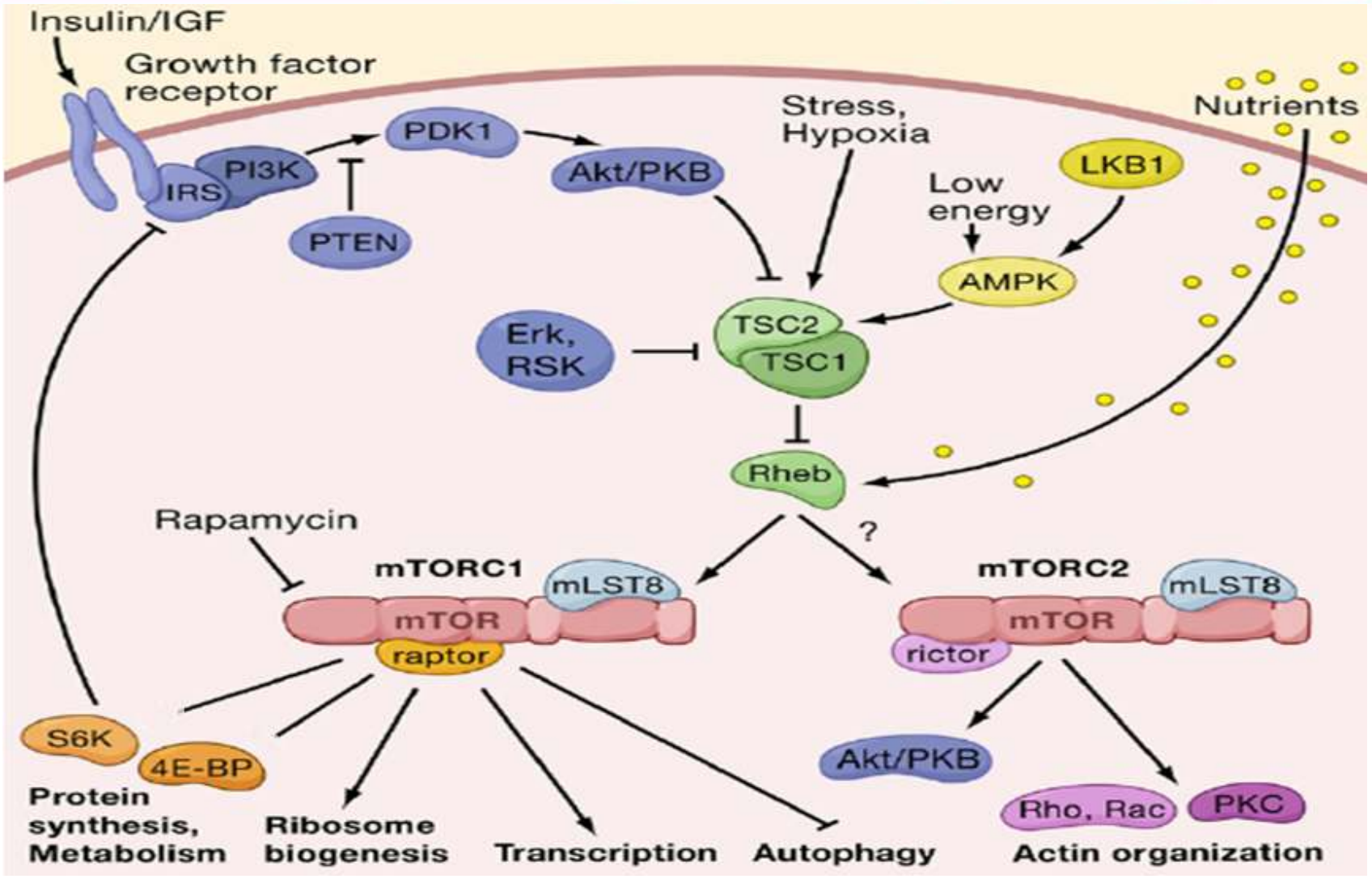
GRANT 006_FC_2015

Background

- **mTOR** kinase cascade serves as a signal integrator of several upstream signals, including growth factors, nutrients, energy levels, and stress.
- In response to such stimuli, the mTOR kinase cascade positively or negatively influences cellular growth and survival
- mTOR kinase cascade can also control T cell differentiation, given that it is capable of facilitating commitment of naïve T cells along the Th1/Th17 pathways and inhibiting the differentiation of T cells into regulatory T cells
- The hyper-activation of mTOR occurs in many immune-inflammatory diseases, also involving the intestine
- Altogether these data suggest that mTOR kinase cascade may be involved in the inflammatory response in CD

Peter et al. Curr Opin Immunol. 2010
Long et al. J Dig Dis. 2013
Yang et al. J Cell Physiol. 2014
Byles et al. Nat Commun. 2013

GRANT 006_FC_2015



Wullschleger et al. Cell 2016

Aim of the study

To investigate the contribution of mTOR kinase cascade in the CD-related mucosal inflammation

Methods (1)

- ✓ Duodenal biopsies of patients with active CD (ACD) on a gluten-containing diet, patients with inactive CD (ICD) on a gluten-free diet and normal controls (CTR)
- ✓ p-mTOR was evaluated in duodenal sections by immunohistochemistry, and total proteins extracted from biopsy samples were analyzed for p-4EBP, p-p70S6k and p-Rictor by Western blotting (WB)
- ✓ Analysis of mTOR inhibitors, such as TSC1 and TSC2, was performed by real-time PCR

Methods (2)

- ✓ Mucosal explants of active CD patients were cultured with wortmannin, an inhibitor of Akt, and the expression of p-Akt, p-mTOR, p-4EBP e p-p70S6K was evaluated by WB
- ✓ Mucosal explants of inactive CD patients were treated with peptic-tryptic digest of gliadin (PT) and expression of p-4EBP e p-p70S6K was evaluated by WB
- ✓ Normal intraepithelial lymphocytes (IELs) isolated from normal jejunal specimens were stimulated with inflammatory cytokines over-produced in active CD (i.e. IFNγ, IL-15, IL-17A and IL-21) and expression of p-4EBP was evaluated by WB

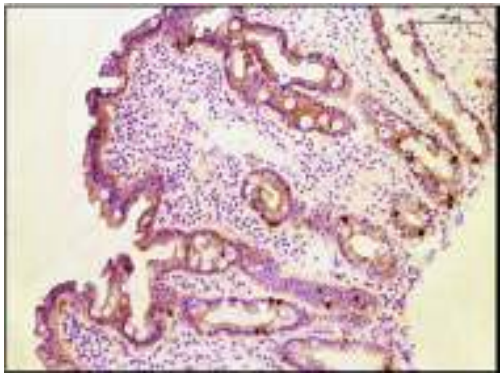
Specific aim (1)

To evaluate expression of active mTOR in active celiac disease

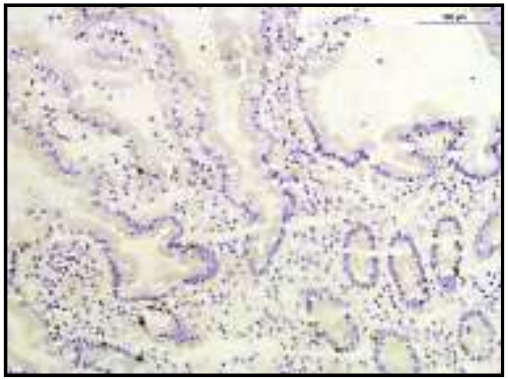
Results

Expression of active mTOR is increased in active CD

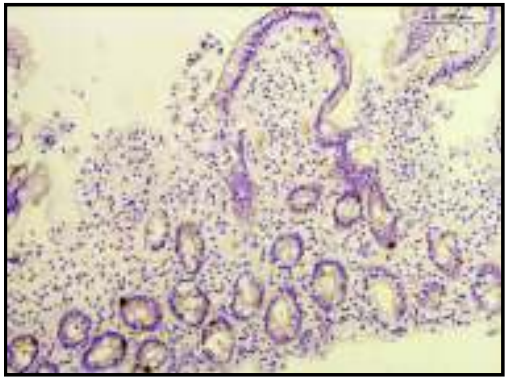
ACD



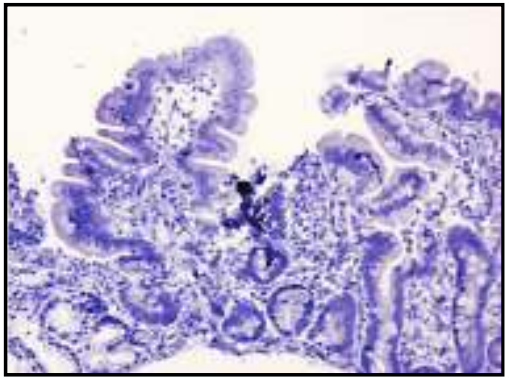
CTR



ICD



NEG

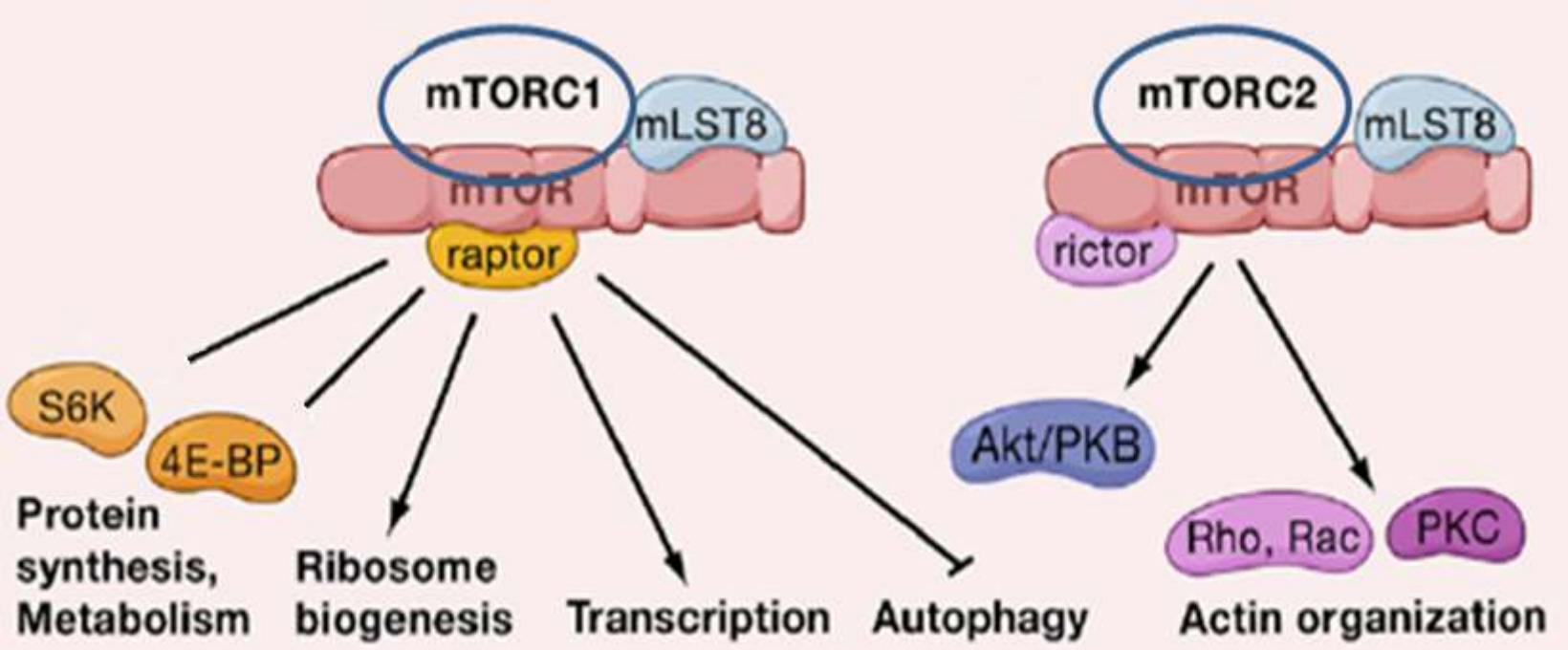


No difference in the expression of active mTOR between ICD and CTR

Specific aim (2)

To examine the involvement of mTORC1 or mTORC2 in active celiac disease

Background

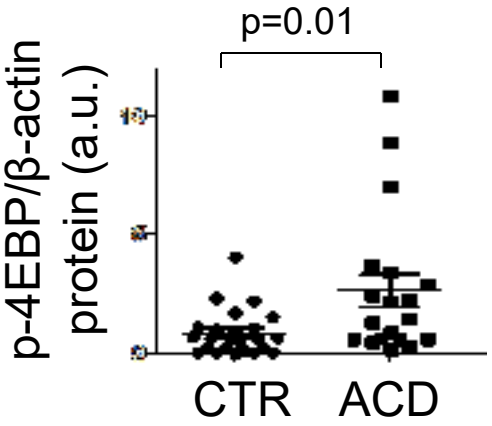


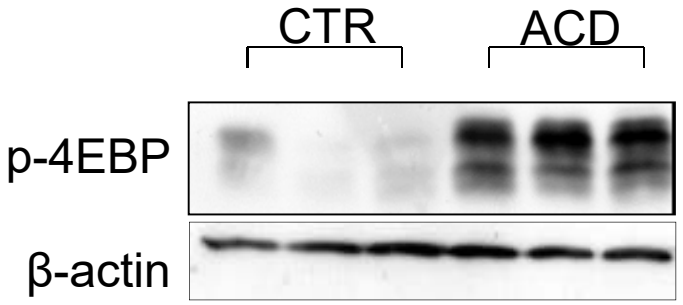
Wullschleger et al. Cell 2016

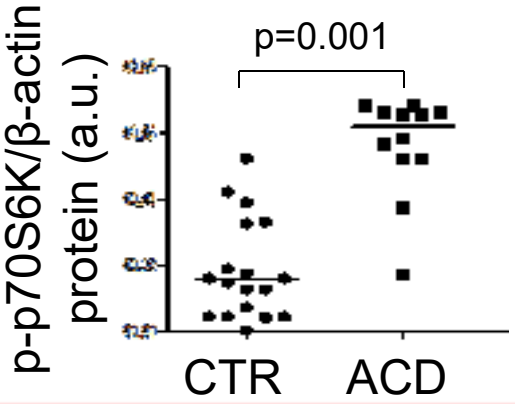
GRANT 006_FC_2015

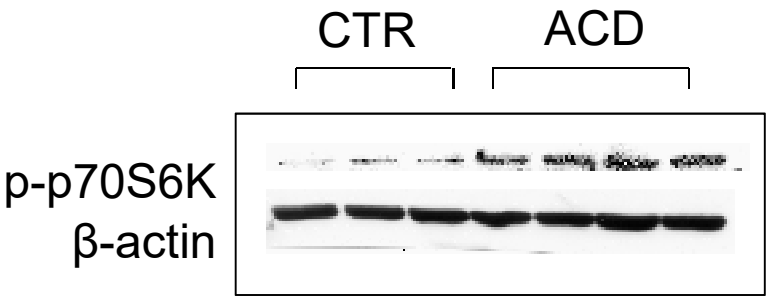
Results

Expression of phosphorylated 4-EBP and p70S6K, two down-stream targets of mTORC1, is increased in active CD



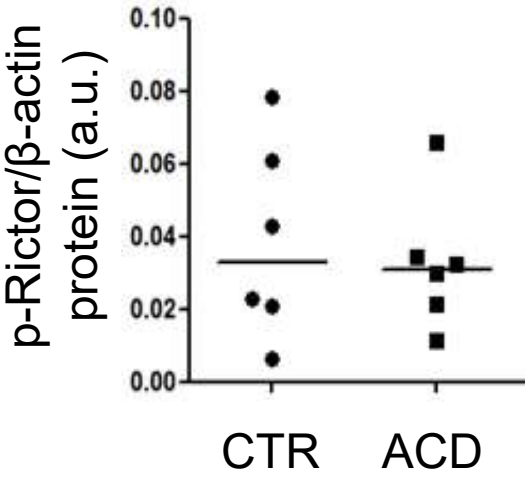


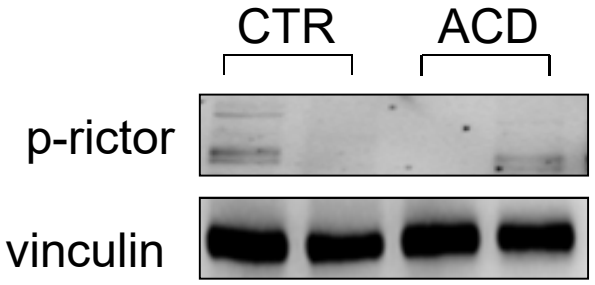




Results

Expression of phosphorylated rictor, a component of mTORC2, is not up-regulated in active CD

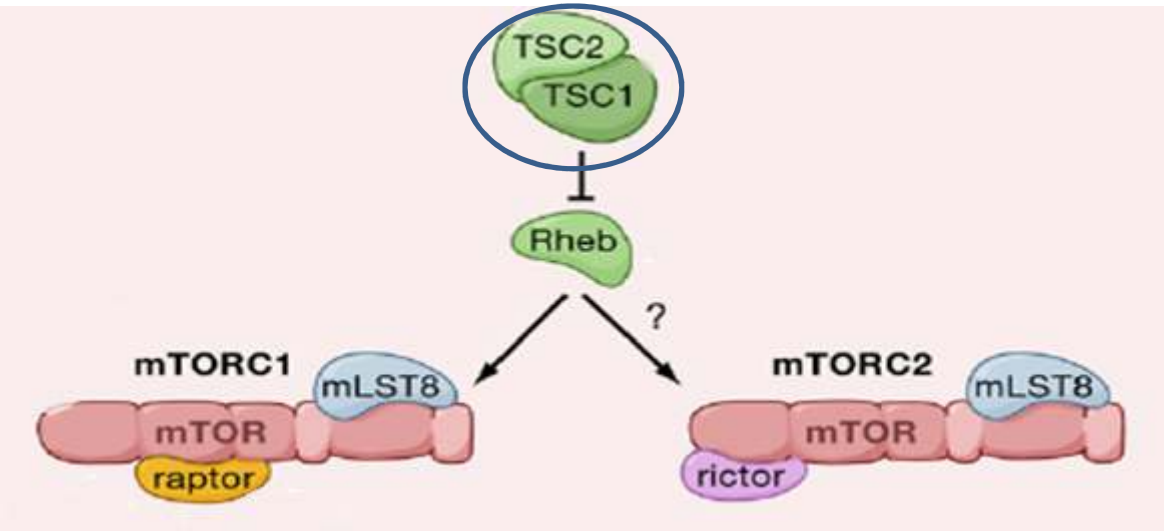




Specific aim (3)

To evaluate whether activation of mTOR in active celiac disease associates with changes in the expression of mTOR signaling inhibitors

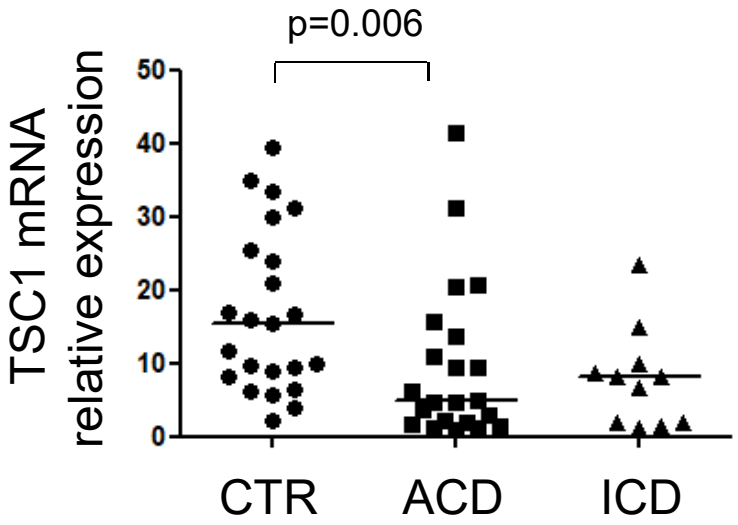
Background

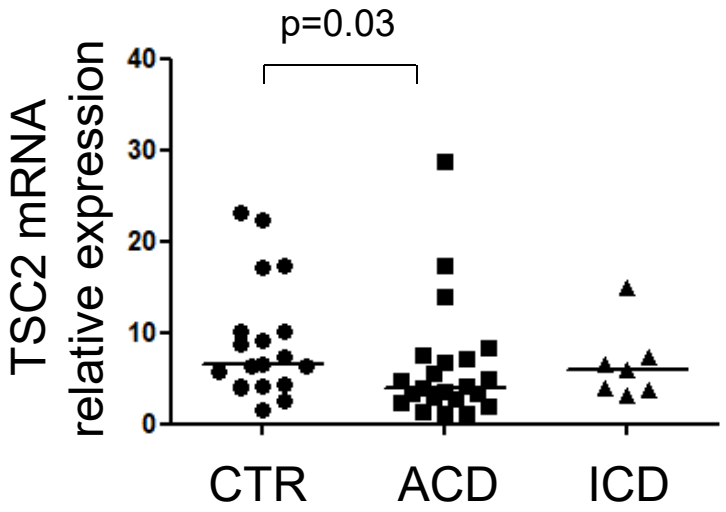


Wullschleger et al. Cell 2016

Results

RNA-transcripts of TSC1 and TSC2, two inhibitors of mTOR, are reduced in active CD

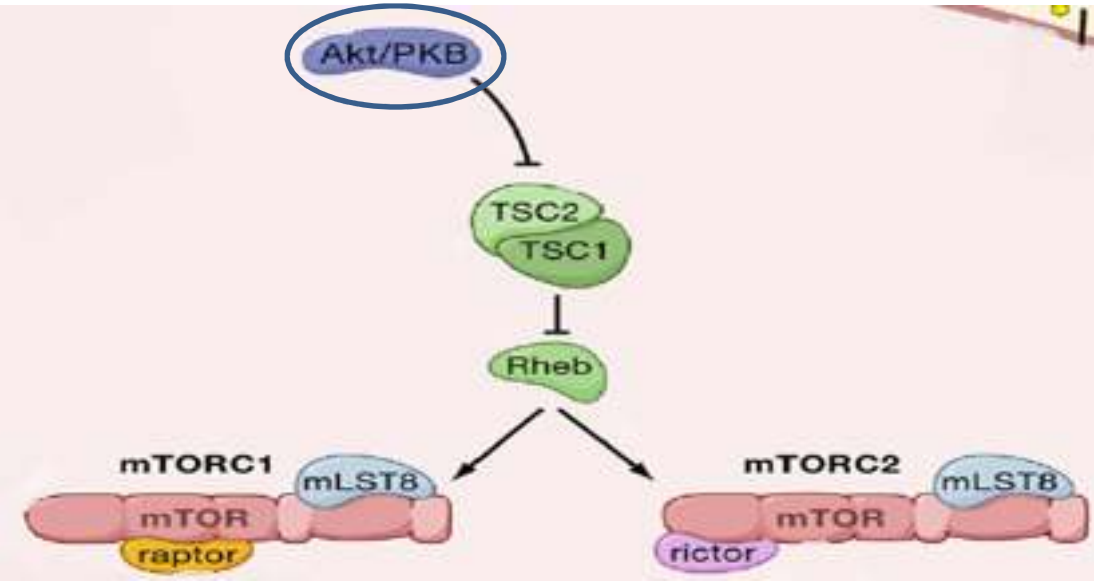




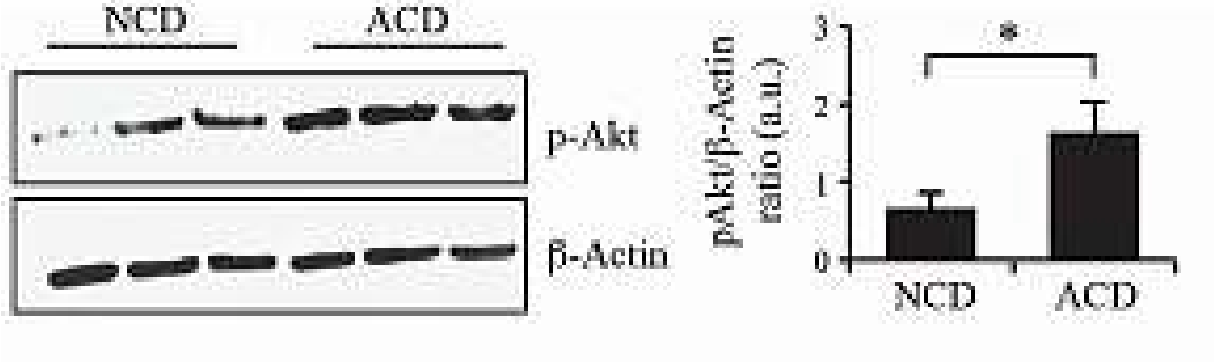
Specific aim (4)

To determine which factors/mechanims sustain activation of mTOR in active CD

GRANT 006_FC_2015



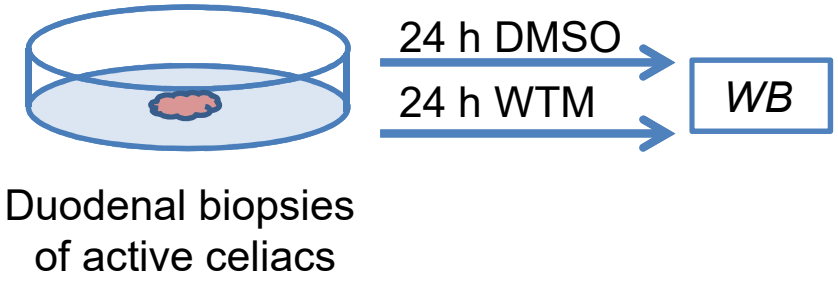
Previous studies have shown that Akt, a kinase highly expressed in the duodenal mucosa of celiacs, can positively regulate mTOR activation

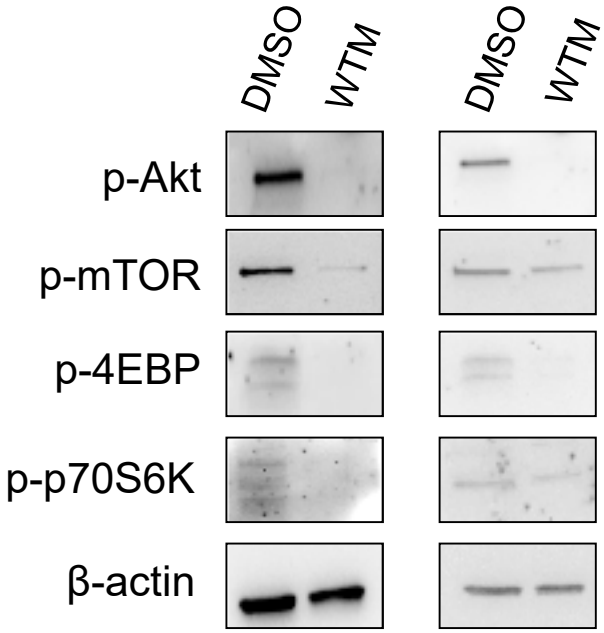


Wullschleger et al. Cell 2016
Sarra et al. Mucosal Immunol. 2013

Results

Inhibition of Akt with wortmannin decreases p-4EBP and p-p70S6K expression in active CD mucosa



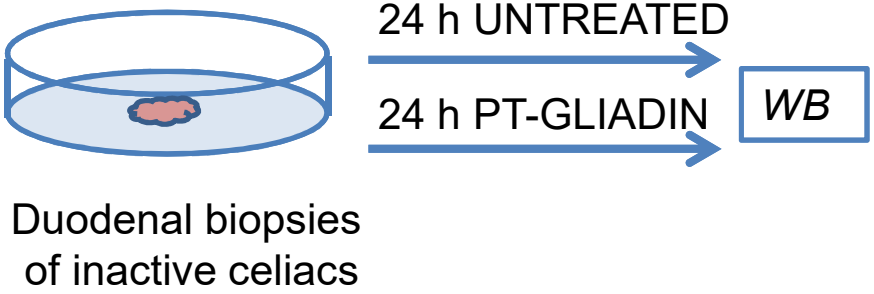


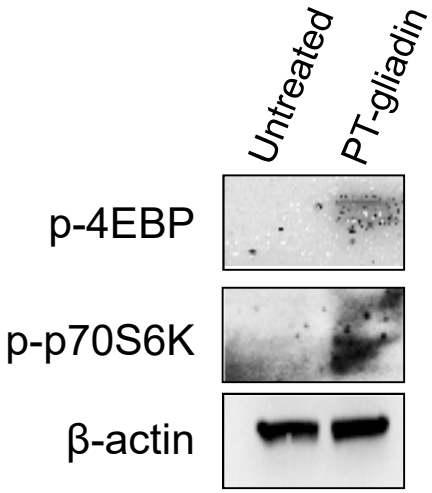
Specific aim (5)

To assess if mTOR is activated by gliadin

Results

Treatment of duodenal samples taken from inactive CD patients with peptic-tryptic digest of gliadin increases p-4EBP and p-p70S6K



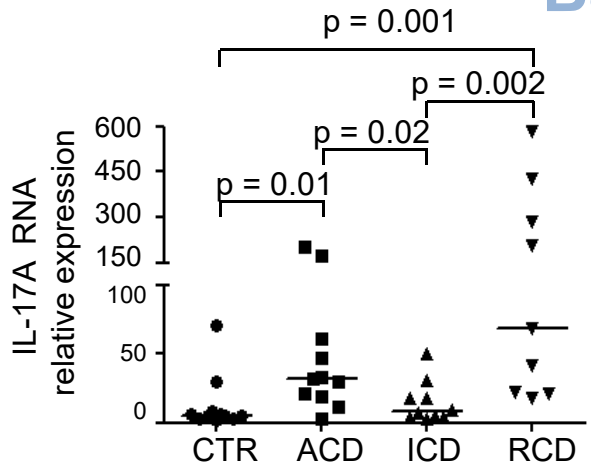


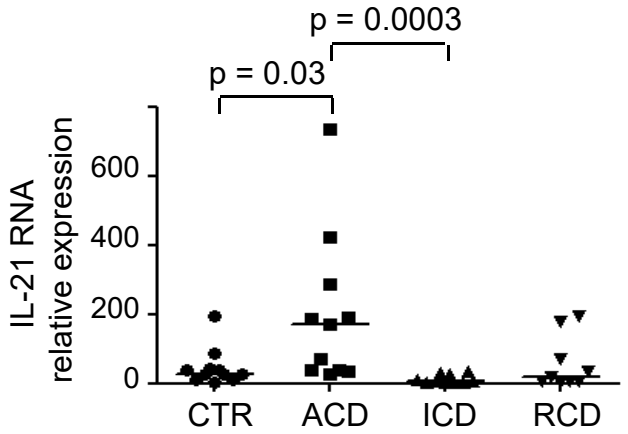
Specific aim (6)

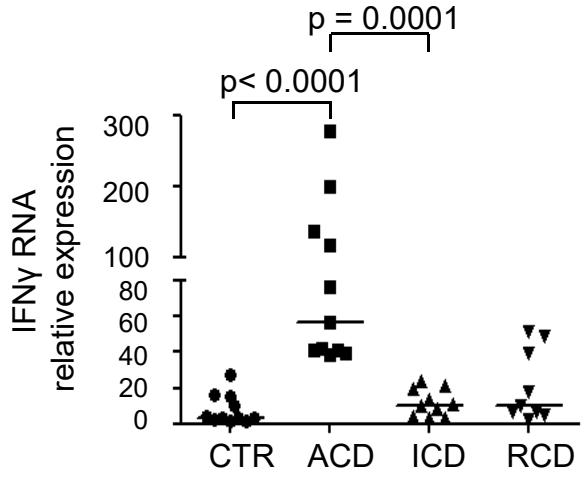
To determine whether inflammatory cytokines over-produced in the duodenum of active celiac disease patients induce the expression of p-4EBP

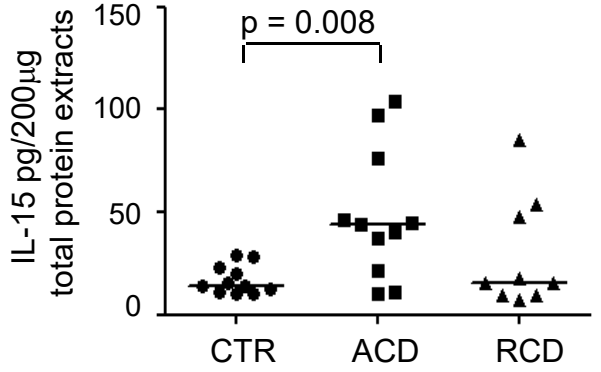
GRANT 006_FC_2015

Background





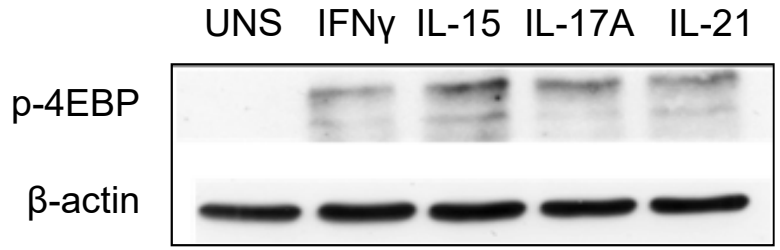




Caruso et al. Clin Sci 2014

Results

p-4EBP expression increases after stimulation of normal IELs with inflammatory cytokines



Summary (1)

- Expression of active mTOR is increased in active CD
- Expression of phosphorylated 4-EBP and p70S6K, two down-stream targets of mTORC1, is increased in active CD
- RNA-transcripts of TSC1 and TSC2, two inhibitors of mTOR, are reduced in active CD

Summary (2)

- Inhibition of Akt with wortmannin decreases p-4EBP and p-p70S6K expression in active CD mucosa

- Treatment of duodenal samples taken from inactive CD patients with peptic-tryptic digest of gliadin increases p-4EBP and p-p70S6K

- p-4EBP expression is enhanced in normal IELs by inflammatory cytokines

Conclusion

mTOR signaling is highly activated in CD

Next plan

- To confirm the preliminary data using additional samples of active CD, inactive CD and controls
- To evaluate mTOR signaling in other pathologies (i.e. Crohn's disease, GVHD, infectious diseases)
- To evaluate mTOR signaling in organ culture of ACD or ICD patients after stimulation with neutralizing antibodies or cytokines
- To evaluate mTOR signaling and the expression of transcription factors in normal LPMC and IELs after stimulation with inflammatory citokynes
- To evaluate mTOR signaling in refractory celiac disease


thank
you