Modulators of the interaction of Astrin and Raptor, and uses thereof in cancer therapy

Technology

Mammalian target of rapamycin complex 1 (mTORC1) controls growth and survival in response to metabolic cues. Oxidative stress affects mTORC1 via inhibitory and stimulatory inputs. Whereas downregulation of TSC1-TSC2 activates mTORC1 upon oxidative stress, the molecular mechanism of mTORC1 inhibition remains unknown. We identified Astrin as an essential negative mTORC1 regulator in the cellular stress response. Upon stress, Astrin inhibits mTORC1 association and recruits the mTORC1 component Raptor to stress granules (SGs), thereby preventing mTORC1-hyperactivationinduced apoptosis. In turn, balanced mTORC1 activity enables expression of stress factors. By identifying Astrin as a direct molecular link between mTORC1, SG assembly, and the stress response, we establish a unifying model of mTORC1 inhibition and activation upon stress. **Importantly, we show that in cancer cells, apoptosis suppression during stress depends on Astrin. Being frequently upregulated in tumors, Astrin is a potential clinically relevant target to sensitize tumors to apoptosis.**

Innovation

- Astrin-Raptor (mTORC1) interaction modifies apoptosis susceptibility of cancer cells

Application

- Targeting Astrin-Raptor interaction may be a relevant approach for drug development

Market Potential

- The Astrin gene is amplified in a large number of tumors (cBIO database), including breast and non-small cell lung cancer. The mTORC1 network is altered in over 70% of all tumors (cBIO data base). Thus, many patients may be amenable to Astrin-Raptor based therapies.

Thedieck K, Holzwarth B et al. Inhibition of mTORC1 by Astrin and stress granules prevents apoptosis in cancer cells. **Cell (2013)** 15 Aug; 154 (4):859-874

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Inhibition of mTORC1 by Astrin and stress granules prevents apoptosis in cancer cells

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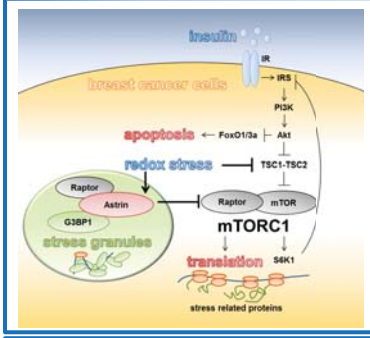
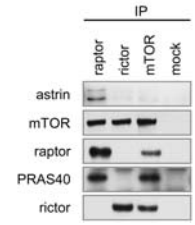
Summary

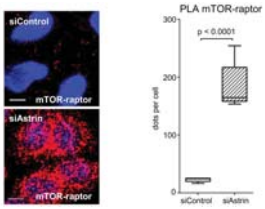


- Mammalian target of rapamycin complex 1 (mTORC1) controls growth and survival in response to metabolic cues. Oxidative stress affects mTORC1 via inhibitory and stimulatory inputs. Whereas downregulation of TSC1-TSC2 activates mTORC1 upon oxidative stress, the molecular mechanism of mTORC1 inhibition remains unknown.
- We identify astrin as an essential negative mTORC1 regulator in the cellular stress response.
- Upon stress, astrin inhibits mTORC1 association and recruits the mTORC1 component raptor to stress granules (SGs), thereby preventing mTORC1-hyperactivation-induced apoptosis. In turn, balanced mTORC1 activity enables expression of stress factors.
- By identifying astrin as a direct molecular link between mTORC1, SG assembly, and the stress response, we establish a unifying model of mTORC1 inhibition and activation upon stress. Importantly, we show that in cancer cells, apoptosis suppression during stress depends on astrin.
- Being frequently upregulated in tumors, astrin is a potential clinically relevant target to sensitize tumors to apoptosis.

1. Astrin Binds to Raptor and Prevents mTORC1 Assembly

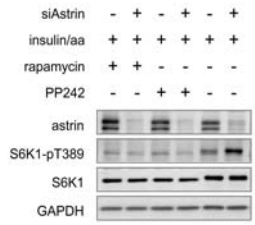


Astrin coimmunoprecipitates with raptor but not with mTOR or the mTORC2 component rictor.

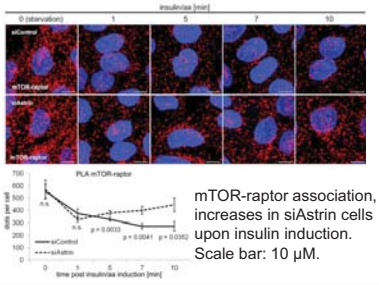


Astrin knockdown enhances mTOR-raptor association, as shown by proximity ligation assay (PLA). Scale bar: 10 µM.

2. Astrin Deficiency Induces mTORC1

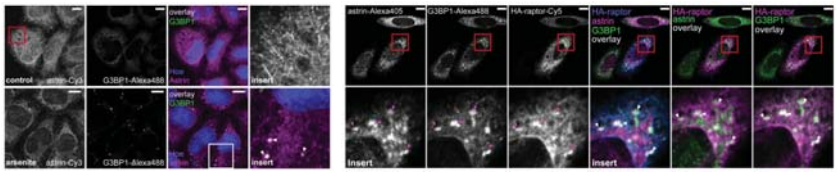


Astrin knockdown induces mTORC1 activity.



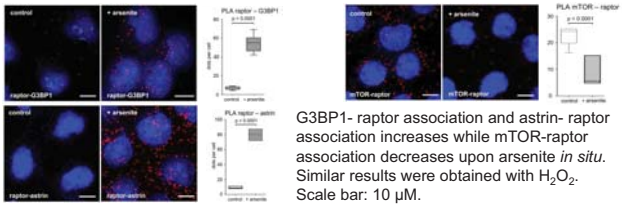
mTOR-raptor association, increases in siAstrin cells upon insulin induction. Scale bar: 10 µM.

3. Astrin and Raptor Relocalize to SGs upon Oxidative Stress



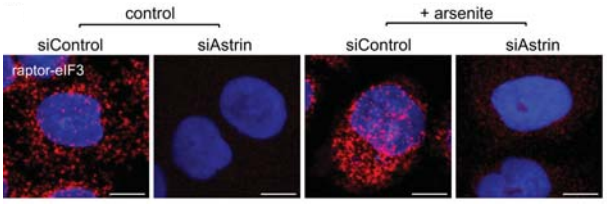
HA-raptor colocalized with astrin and G3BP1 upon oxidative stress (arsenite).

4. Oxidative Stress Induces Astrin-Raptor Association in SGs and Reduces Raptor-mTOR association

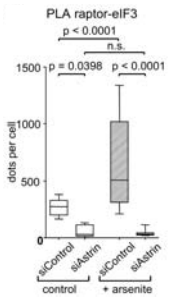


G3BP1- raptor association and astrin- raptor association increases while mTOR-raptor association decreases upon arsenite *in situ*. Similar results were obtained with H_2O_2. Scale bar: 10 µM.

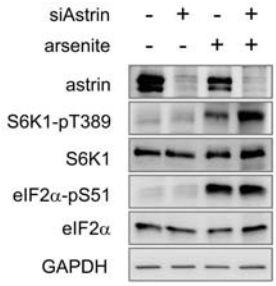
5. Astrin Is Required for Raptor Recruitment to SGs upon Redox Stress



The raptor-SG association upon oxidative stress is astrin dependent. eIF3, SG marker; scale bar: 10 µM.

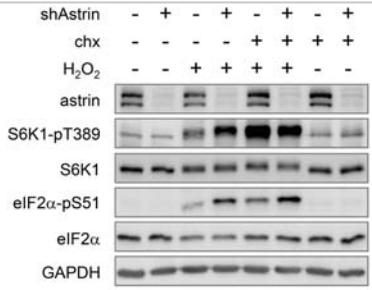


6. Astrin Inhibits mTORC1 under Oxidative Stress

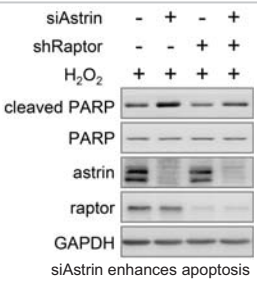


Astrin inhibition further induces S6K1-T389 phosphorylation upon arsenite. Similar results were obtained with H_2O_2.

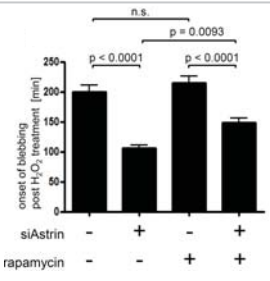
7. SGs Are Required for mTORC1 Inhibition by Astrin under Oxidative Stress, and SG and Astrin-Dependent mTORC1 Repression Protects Cells from Oxidative Stress-Induced Apoptosis



Inhibition of SG assembly by cycloheximide (chx) combined with oxidative stress (H_2O_2) strongly induces S6K1-pT389, and under these conditions, shAstrin fails to further induce mTORC1 activity.



siAstrin enhances apoptosis upon H_2O_2 treatment.



Rapamycin suppressed apoptosis in siAstrin cells, as confirmed by live-cell imaging to determine the onset of apoptotic membrane blebbing.

Reference

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