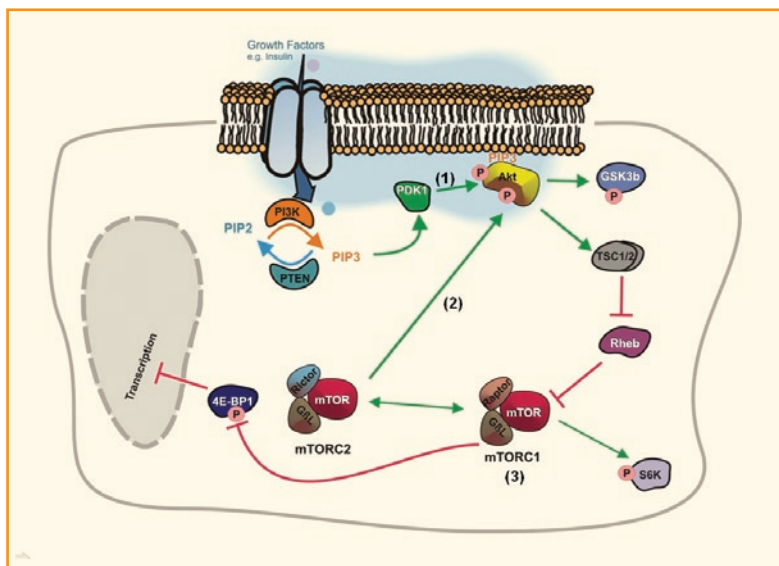


KINAXO's Cellular Target Profiling® reveals mTOR as a new target for celecoxib

Summary

- Cellular Target Profiling® of celecoxib (Celebrex®/Pfizer) identified the protein kinase mTOR as a new target. mTOR acts as a central regulator of cell proliferation, cell survival, angiogenesis and cell metabolism, and is a key intracellular convergence point for a number of signaling pathways that are abnormally activated in many types of cancer.
- Celecoxib is a non-steroidal, anti-inflammatory Cox-2 inhibitor marketed by Pfizer and approved for the treatment of osteoarthritis, rheumatoid arthritis and acute pain. Celecoxib also shows anti-proliferative effects and is currently being tested in numerous clinical trials against several malignancies. However, its anti-cancerogenic effects are poorly understood.
- KINAXO Cellular Target Profiling® is a highly effective method to (re-)profile small molecules. It can reveal new target proteins and thereby increase our understanding of a drug's cellular mode of action. This can also help identify new application areas for already approved drugs.

The role of mTOR signaling in cell proliferation and survival



Growth factors bind to their cognate receptor tyrosine kinases (RTK), which trigger PI3K activation to generate PIP3 (phosphatidylinositol 3,4,5-trisphosphate). PIP3 leads to activation of PDK1, which in turn phosphorylates Thr308 of Akt (1).

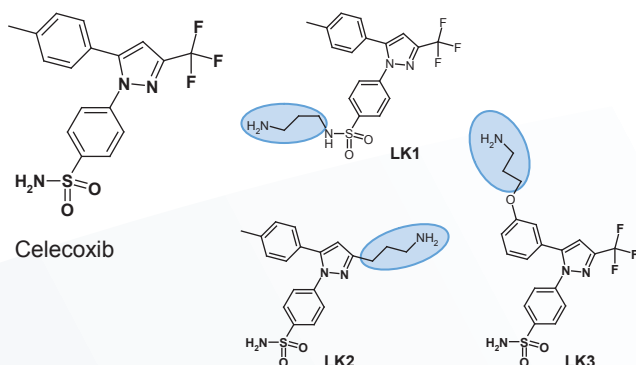
mTOR, when bound to Rictor (rapamycin-insensitive companion of mTOR), specifically phosphorylates Akt at Ser473 in a growth factor dependent manner (2).

Akt, a crucial regulator of cell proliferation and survival pathways, is hyper-activated in many tumors. Inhibitors that interfere with Akt activation are therefore highly sought for the treatment of cancer.

In contrast to the mTOR-Rictor complex, mTOR in a complex with the regulatory associated protein of mTOR (Raptor) does not phosphorylate Akt, but influences translation by phosphorylation of the elongation factor 4E-BP and the ribosomal protein kinase S6 (p70S6K) (3).

KINAXO's Cellular Target Profiling® of celecoxib

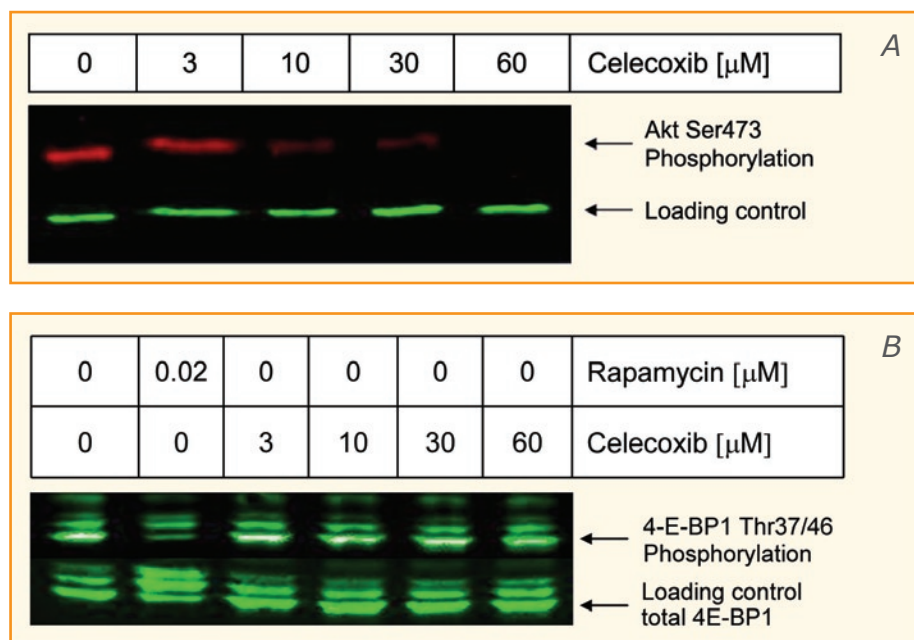
Target profiling experiments were performed using affinity chromatography in combination with state-of-the-art quantitative mass spectrometry (for further information see **TN1**). Chemical linkers for immobilization were added at three different sites to enable celecoxib to interact with its protein targets via different binding mechanisms.



Chemical structure of celecoxib and its derivatives used for KINAXO Cellular Target Profiling®

Three different linker compounds (LK1-LK3) were generated and coupled to a sepharose matrix to capture cellular protein targets of celecoxib. mTor binds to this immobilized matrix in a concentration dependent manner.

Validation of mTOR/Rictor as a target protein of celecoxib



A) Phosphorylation of Akt Ser473, which is known to be specific for the mTOR-Rictor complex, can be eliminated by addition of celecoxib.

B) Cellular assays showed no effect of celecoxib on downstream target phosphorylation of the mTor-Raptor complex, which demonstrates that the drug probably only has an effect on the mTOR-Rictor complex. In an *in vitro* kinase assay celecoxib showed no effect on the kinase activity of mTor (data not shown). All experiments were performed in PC3 (human prostate cancer) cells.

References

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- (2) Sarbassov *et al.*, Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex, *Science*, 2005, **307**(5712):1098-101.
- (3) Guertin *et al.*, Defining the role of mTOR in cancer, *Cancer Cell*, 2007, **12**(1):9-22.

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