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TITLE
LKB1 stabilizes the cRTC1 protein through a TNPO1-dependent mechanism

HYPOTHESIS:
We have observed that in lung cancer samples and cells, the LKB1 presence related to the accumulation of CRTC1 protein. However, the fact that the RNA level don’t increasing suggests that this accumulation is about the stability of CRTC1. We aim to study the mechanism underlying CRTC1 stability.

BACKGROUND/AIMS:
CRTCAs are a family of CREB coactivators that regulate ER stress, glucose/fatty acid metabolism, lifespan prolongation in murine and C.elegans models, and cancer development. CRTCAs activation is controlled by nuclear cytoplasmic shuttling but the nuclear import mechanism is unknown. LKB1 is the susceptibility locus for the Peutz-Jeghers cancer syndrome and LKB1 mutation is among the most common somatic alterations in lung cancer. AMP – activated protein kinase (AMPK) is a sensor of cellular energy status. It is activated, by a mechanism requiring the tumor suppressor LKB1. Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) show indisputable promise as chemotherapy agents by the general mechanism of directly activation of AMPK. We previously showed that aberrant phosphorylation and activation of CREB-regulated transcription co-activator 1 (CRTC1) exclusively in non-small cell lung cancer (NSCLC) samples carrying LKB1 null mutations. Here we provide the evidence that Aspirin and Salicylate can stabilize CRTC1 protein through LKB1 underlying a mechanism of TNPO1.

METHODS:
Somatic LKB1 mutations are present in 20% of lung adenocarcinomas and we observed degradation of CRTC1 in LKB1-null lung adenocarcinomas samples, but the accumulation of CRTC1 protein in LKB1-wildtype tissues. To study the mechanism underlying CRTC1 degradation, we employed yeast two-hybrid, immunoprecipitation of GST-fusion recombinant proteins, and mass spectroscopy analysis of peptides immunoprecipitated by anti-CRTC1 in large-scale cell lysates and identified transportin-1 as a candidate protein import binding partner. To validate these finding, we screen the CRTC1 amino acid sequence in different species. We found the similar cysteine mutation pattern of CRTC1 in different species which are crucial for TNPO1 binding. We construct CRTC1 recombinants by mutate the cysteines in CRTC1 which are C131 and C216. The immunoprecipitation assay shows that the c131 is important for CRTC1 stability. Further study by the western blot of protein extract from cycloheximide treated cells shows that the stabilization of LKB1 to CRTC1 can be blocked by cycloheximide. The main mechanism underlying the beneficial effects of Aspirin reducing risk of cancers has been issued as direct activation of AMPK. Here we provide the evidence that Aspirin and Salicylate activate AMPK and enhance the expressing of CRTC1 by LKB1.

RESULTS & CONCLUSIONS
Somatic LKB1 mutations are present in 20% of lung adenocarcinomas and we observed degradation of CRTC1 in LKB1-null lung adenocarcinomas samples, but the accumulation of CRTC1 protein in LKB1-wildtype tissues. Further study by the western blot of extract from cycloheximide treated cells shows that the stabilization of LKB1 to CRTC1 can be altered by cycloheximide. By yeast two-hybrid, immunoprecipitation of GST-fusion recombinant proteins, and mass spectroscopy analysis of peptides immunoprecipitated, we identified transportin-1 as a candidate protein import binding partner. To validate these finding, we screen the CRTC1 amino acid sequence in different species. We found the similar cysteine mutation pattern of CRTC1 in different species which are crucial for TNPO1 binding. We construct CRTC1 recombinants by mutate the cysteines in CRTC1 which are C131 and C216. The immunoprecipitation assay shows that the c131 is important for CRTC1 stability. Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) show indisputable promise as chemotherapy agents by the general mechanism of directly activation of AMP – activated protein kinase (AMPK). Here we provide the evidence that Aspirin and salicylate activate AMPK and enhance the expressing of CRTC1 by LKB1.