AMP-Activated Protein Kinase: Regulating Cellular and Whole Body Energy Balance

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AMPK (AMP-activated protein kinase)

- Introduction – AMPK as an energy sensor
- AMPK as a drug target in type 2 diabetes
- Identification of upstream kinases
- Identification of AMP-binding sites
- AMPK as a drug target in cancer

Why a signalling pathway activated by AMP?

Ischaemia

Catabolism

2 ADP → AMP → AMPK → ATP

Contraction

ATP consumption
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Regulation of AMPK by AMP and ATP

Function of AMPK orthologues in lower eukaryotes

- In the budding yeast Saccharomyces cerevisiae:
  - SNF1 complex required for the response to glucose starvation
  - Required for the switch from fermentative to oxidative metabolism
- In the moss Physcomitrella patens, a primitive green plant:
  - Essential for growth in alternate light/dark cycles, although not required for growth in continuous light
- In the nematode worm Caenorhabditis elegans:
  - Required for lifespan extension in response to caloric restriction
- In the insect Drosophila melanogaster:
  - Required for maintenance of cell polarity in the early embryo

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Regulation of metabolism by AMPK activation

Protein synthesis cell growth (mTOR) → Glucose uptake → Glycolysis → AMPK → Fatty acid oxidation

Gluconeogenesis → Cholesterol synthesis → Fatty acid synthesis

Mitochondrial biogenesis

Regulation of AMPK by drugs, cytokines and other stimuli

Exercise → AICAR → Metformin

Adiponectin → AMPK

Leptin (hypothalamus) → Leptin (muscle) → Thiazolidinediones (rosiglitazone, pioglitazone)

A-769662 (Abbott labs)

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Anti-diabetic drugs: phenformin & metformin

- French lilac (goat’s rue) used in middle ages to treat diabetes
- In the 1920s the active component identified as derivative of guanidine
- Biguanide derivatives introduced to clinical use in the 1950s
- Metformin now prescribed to >120 million type 2 diabetics world-wide
- In 2001, metformin shown to activate AMPK

Phytochemicals from foods, beverages, traditional medicines

- Resveratrol (red wine, chocolate) (extends lifespan)
- Berberine (from Berberis spp.) (anti-diabetes effect)
- Epigallocatechin 3-gallate (EGCG, green tea) (anti-cancer, obesity, diabetes effect)
- Genistein (soybean: anti-cancer effect)

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Regulation of AMPK by AMP and ATP

The budding yeast kinome

Activation of AMPK by yeast kinases

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The budding yeast kinome

- **SNF1 group**
  - SNF1
  - SNF1-related kinases

- **NPR/HAL group**
  - NPR1
  - HAL5
  - YDL025C

- **CAMK group**
  - CAMK1
  - CAMK2

- **CMGC group**
  - CMGC1
  - CMGC2

- **AGC group**
  - AGC1
  - ITP1

- **YPR group**
  - YPR106W

- **GIN4 group**
  - GIN4

- **SNF1 group**
  - SNF1
  - SNF1-related kinases

The human kinome

- **LKB1**
- **AMPK**
- **CaMKK**

**LKB1 - a tumour suppressor**

- LKB1 identified in 1998 as gene mutated in the human cancer syndrome, Peutz-Jeghers syndrome, inherited as an autosomal dominant mutation.
- Peutz-Jeghers syndrome subjects have numerous benign intestinal tumours, and a 15- to 20-fold increased risk of malignant tumours.
- LKB1 gene often mutated in spontaneous cancers, such as lung adenocarcinomas and cervical cancers.
- LKB1 expression switched off in tumour from which HeLa cells derived.
- LKB1 was a protein kinase, but its downstream targets unknown.
- With Dario Alessi, we showed the major form of AMPKK purified from rat liver was a complex between LKB1 and two accessory subunits, STRAD and MO25, and that knocking out LKB1 prevents AMPK activation. (Hawley et al., 2003, J. Biol. 2: 26)
More upstream kinase - CaMKKs

- In HeLa cells (which lack LKB1) AMPK is still phosphorylated, and this is increased by Ca²⁺ ionophores.
- Effect blocked by STO-609, a CaMKK inhibitor.
- Effect also reduced by siRNA knockdown of CaMKKβ, but not CaMKKα.
- The calmodulin-dependent protein kinase kinase, CaMKKα and CaMKKβ, also activate AMPK in cell-free assays, although CaMKKβ is more active.
- This Ca²⁺→CaMKKβ pathway is activated:
  - Neurones, where it is activated by depolarization
  - Endothelial cells, where it is activated by Gq-coupled agonists like thrombin
  - T cells, where it is activated by stimulation of the T cell receptor.

Upstream kinases acting on AMPK

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Domain structure of AMPK

Mutations in γ2 CBS motifs cause heart disease

- The common effect of the nine mutations is ventricular pre-excitation (Wolff-Parkinson-White syndrome) with increased risk of fatal arrhythmias
- Milder forms (7) have adult onset with autosomal dominant inheritance
- More severe forms (2) cause infant death from cardiac/respiratory problems
- Post mortem analysis of cardiac tissue shows abnormal glycogen accumulation
- Five of the mutations (R302Q, H383R, R384T, R531G, R531Q) neutralize the positive charge on basic residues in similar positions in CBS1, CBS2 and CBS4
- These mutations interfere with the binding of AMP and ATP, suggesting that these residues directly interacted with the nucleotide phosphate groups


γ2 mutations reduce AMP and ATP binding

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Mutated residues are involved in AMP binding


AMP (non-exchangeable)

Mutated residues are involved in AMP binding (2)


AMP (exchangeable)

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AMPK activators: potential anti-cancer drugs

- The upstream kinase that switches on AMPK, LKB1, is a tumour suppressor
- AMPK activation in tumour cells causes inhibits cell growth and the cell cycle
- Like rapamycin (undergoing cancer trials), AMPK inhibits the mTOR pathway
- Diabetics treated with metformin have 30% lower incidence of cancer than those on other medications
- AMPK activators reduce tumour formation in mouse models

AMPK activators protect PTEN+/- LKB1fl/fl mice


AMPK activation is reduced in breast tumours

- Reduced pAMPK staining was seen in 318/349 tumours [91%]
- pAMPK staining correlated with pACC staining (p<0.01)
- pAMPK staining correlated inversely with histological grade and axillary node metastasis, both indicators of poor prognosis
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AMP-activated protein kinase – conclusions

- AMPK is a cellular energy sensor activated by increases in AMP:ATP ratio
- By responding to adipokines like leptin and adiponectin, AMPK also regulates energy balance at the whole body level
- AMPK has a catalytic α subunit and regulatory β and γ subunits, and is only active after phosphorylation at Thr-172 on the α subunit
- AMP binds to 2 sites on the γ subunit, inhibiting dephosphorylation of Thr-172 and also causing allosteric activation, yielding >1000-fold activation overall
- The major kinase phosphorylating Thr-172 is the tumour suppressor, LKB1
- AMPK is a target for existing anti-diabetic drugs such as metformin, and is under investigation as a drug target in cancer

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