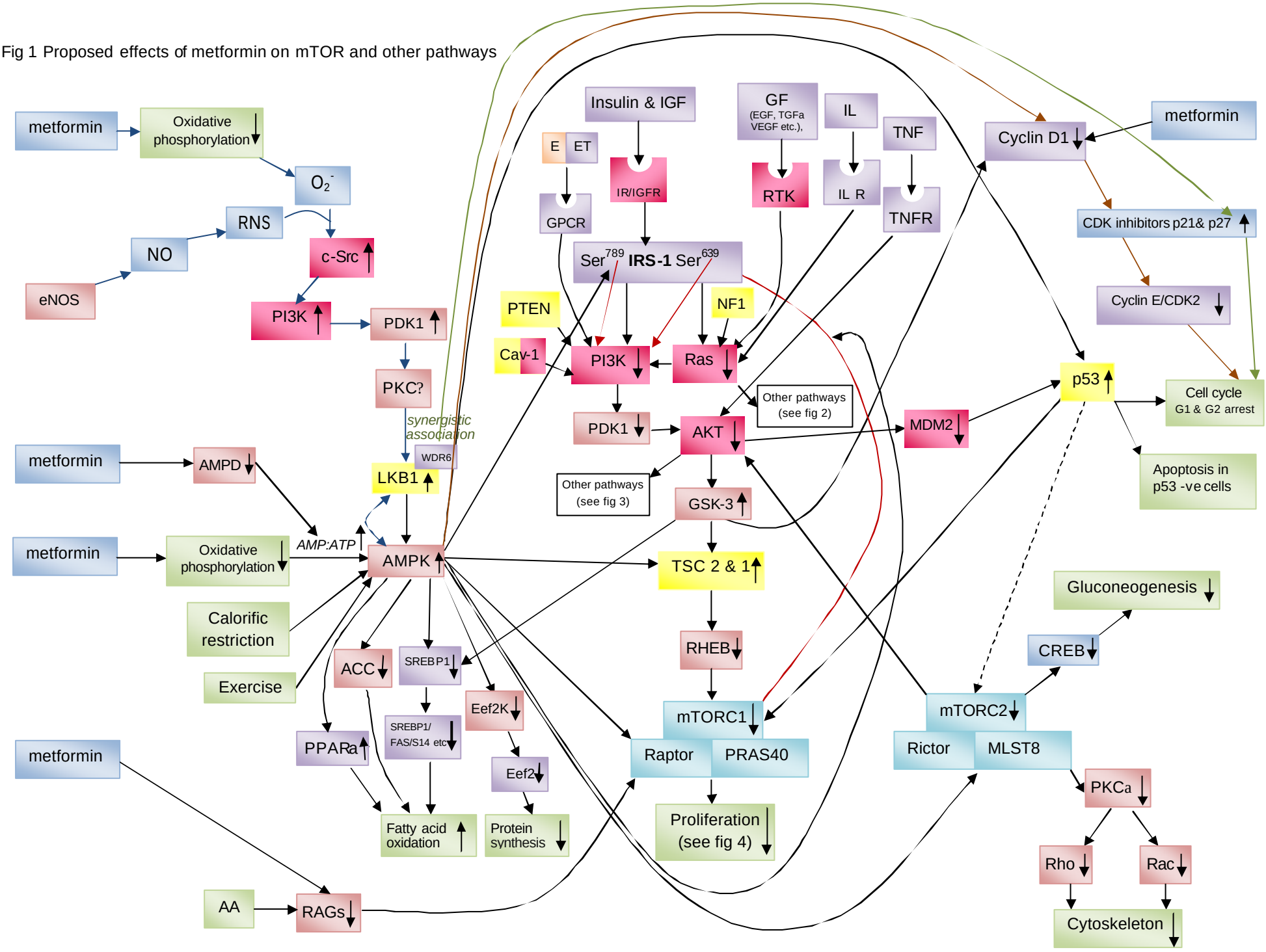
Fig 1 Proposed effects of metformin on mTOR and other pathways



References (In text)

1. Cantrell 2010
2. Chong 2009
3. Grant 2007
4. Isoda 2006
5. Kallender 2010
6. Lui 2009
7. Nakashima 2002
8. Napal 2005
9. Okada 2004
10. Ouyang 2010
11. Ravnskjaer 2006
12. Shackleford 2009
13. Shaw 2005
14. Shaw 2006
15. Xie 2007
16. Xie 2008
17. Zakakini 2010 (feedback loop)
18. Zhou 2001
19. Zhuang 2008
20. Zou 2003

See also below with notes

Legend

↑ Increasing activity

↓ Decreasing activity

In red – activating oncogenic mutations

In yellow – inactivating tumour suppressor mutations

In pink – kinases

In purple proteins

In orange sterols

Abbreviations

AA – amino acids
ACC - acetyl-CoA carboxylase
AKT - serine/threonine protein kinase
AMPD – AMP deaminase
AMPK – AMP-activated protein kinase
CDK - cyclin dependent kinase
CREB - cAMP-response element binding protein
E – oestrogen
eEF2 – Eukaryotic elongation factor -2
eEF2K - Eukaryotic elongation factor -2 kinase
eNOS – Endothelial NO synthase
ET - endothelin
GPCR – G protein coupled receptor
GSK-3 - glycogen synthase kinase-3
IRS-1 – insulin receptor substrate 1 (Ser^{789} - inhibitory site of IRS-1)
LKB1 - serine–threonine kinase liver kinase B1
MDM2 - murine double minute protein
mTORC1 & 2 – mammalian target of Rapamycin complex 1 & 2 (C1 controls cell growth and proliferation; C2 controls cytoskeleton and gluconeogenesis through CREB action on genes)
NF1 - neurofibromin 1
p53 – protein 53 – tumour suppressor (can induce growth arrest by holding the cell cycle at the G_1/S regulation point)
PDK1 - 3-Phosphoinositide dependent protein kinase 1
PI3K - phosphatidylinositol 3-kinase
PKCa - Protein kinase C, alpha
PKC? - Protein kinase C, zeta
PPAR – peroxisome proliferator activated receptor
PTEN - phosphatase and tensin homologue deleted on chromosome 10
Rac - small GTPase
RAGs – RAG GTPases
RAS- small GTPase
RHEB – Ras homologue enriched in brain – GTPase
Rho - small GTPase
RTK - receptor tyrosine kinase
S6K1 – ribosomal protein S6 kinase ß1
SREBP1 - Sterol regulatory element binding transcription factor 1
TNF – Tumour necrosis factor
TSC 1 & 2 - Tuberous sclerosis complex 1 & 2 (tumour growth suppressors – regulate mTOR 1)

WDR6 - WD repeat protein 6

Mechanisms

(feedback loops have been omitted to keep the diagram as straightforward as possible apart from the feedback loops to the IRS-1 complex)

1. In blue – metformin activation of LKB1 (Xie 2008) and Zou 2003
2. In red – negative feedback loop where mTOR activation by insulin induces phosphorylation of the inhibitory Ser^{639} site of IRS-1 and therefore limits activation of AKT (Zakakini 2010)
3. In black – metformin inhibits complex 1 of the electron transfer chain leading to an increase in the AMP:ATP ratio which activates AMPK. As AMPK is activated a feedback loop to LKB1 activates increased synthesis of LKB1 (tumour suppressor).
4. metformin's actions on AMPK lead to phosphorylation of inhibitory site Ser789 of IRS-1 leading to inhibition of AKT activation (leading to apoptosis) together with reduced mTOR activation through inhibitory TSC2&1 activation (Zakakini 2010).
5. metformin inhibits RAGs independently of AMPK which lessens RAGs activation of TORC1 through AA input. RAGs direct mTORC1 to a perinuclear area containing RHEB leading to kinase activation (Kallender 2010).
6. metformin inhibits mTORC2 activation through AMPK which prevents mTORC2 nuclear translocation therefore inhibiting gene expression involved in gluconeogenesis by CREB (Chong 2009; Shaw 2005).
7. Activation of AMPK may induce tumour suppression through activation of LKB1 tumour suppressor (Chong 2009 & others). AMPK activation resulted in Cell Cycle G1 arrest through p53 (which controls the cell cycle - ref) and possibly apoptosis also mTORC1 inhibition. Decrease in mTERT (telomerase reverse transcriptase) expression showed decrease in telomerase activity resulting in maintenance of telomere length in cancer cells – vital for proliferation (Cantrell 2010). Jones 2005 – "AMPK activation induces phosphorylation of p53 on serine 15, and this phosphorylation is required to initiate AMPK-dependent cell-cycle arrest."
8. In brown - "metformin arrests cell proliferation by activating AMPK. Active AMPK leads to loss of cyclin D1 mRNA and protein. The decline in cyclin D1 levels causes the release of sequestered CDK inhibitors, $p27^{Kip1}$ and $p21^{Cip1}$, which then bind to and inhibit the cyclin E/CDK2 complex. This prevents progression from G1 into S phase and blocks cell proliferation." (Zhuang 2008)
9. In green - Xie 2007 "WDR6 was able to synergize with LKB1 in cell cycle G1 arrest in Hela cells. Coexpression of WDR6 and LKB1 was able to induce a cyclin-dependent kinase (CDK) inhibitor p27Kip1. Furthermore, the stimulatory effect of LKB1 on p27Kip1 promoter activity was significantly elevated by coexpression with WDR6. Collectively, these results provided initial evidence that WDR6 is implicated in the cell growth inhibitory pathway of LKB1 via regulation of p27Kip1" ((anti-tumour effect of LKB1 enhanced by metformin's induction of AMPK or direct activation of LKBI is further enhanced by WDR6 which acts synergistically in association with LKB1)).
10. Isoda 2006 - Akt also associates directly with IKK, and activates IKK-_ via phosphorylation at Thr-2.
11. Shu 2007 – metformin phosphorylates AMPK which inhibits? ACC leading to fatty acid oxidation. Also Ampk activation inhibits SREB-1 in the nucleus leading to reduced expression of SREB-1, FAS and S14 etc. leading again to fatty acid oxidation (look up SREB etc)) – check Hezel 2008 diagram – is ACC activated or inhibited?
12. Liu 2009 diagram pathway from AKT to MDM2 (murine double minute protein) a negative regulator of p53.
13. Shaw 2006 – GSK-3 (glycogen synthase kinase-3) controls cell cycle through phosphorylation of e.g. cyclin d1 and cyclin e. It phosphorylates transcription factors such as SREBP1 (Sterol regulatory element binding transcription factor 1). Usually phosphorylation by GSK results in impaired function so that deactivation by AKT means enhanced transcription. (Therefore metformin's actions on AMPK result in GSK activation and inhibits transcription.)

14. Buller 2008 – “TSC2 and rapamycin-sensitive mTOR function downstream of GSK-3 to modulate effects of GSK-3 on glucose uptake and GLUT1 expression. GSK-3 therefore suppresses glucose uptake via TSC2 and mTOR and may serve to match energy substrate utilization to cellular growth.”
15. Ozes 2001 – “Tumor necrosis factor (TNF) inhibited insulin-promoted tyrosine phosphorylation of IRS-1 and activated the Akt/protein kinase B serine-threonine kinase, a downstream target for phosphatidylinositol 3-kinase (PI 3-kinase)”. “TNF impairs insulin signaling through IRS-1 by activation of a PI 3-kinase/Akt/mTOR pathway, which is antagonized by PTEN”.
16. Tsou 2010 “TNF-alpha increased iNOS expression and NO production in myoblasts via the ILK/Akt/mTOR and NF-kappaB signaling pathway.” Abs only – myoblasts
17. Dan 2008 “TNF, in most cells examined, activates Akt to use IKKalpha to control mTOR activation. In MCF7 cells, TNF does not activate Akt and requires IKKbeta to activate mTOR. The results show that Akt-dependent signaling, induced by cytokines or insulin, alters the IKK subunit-dependent control of mTOR” leads to inflammation.
18. Huang 2009 - metformin had anti-inflammatory effects on endothelial cells and inhibited TNF-a-induced IKKa/ß phosphorylation, I?B-a degradation and IL-6 production in HUVEC. This effect was related to PI3K-dependent AMPK phosphorylation
19. Huang 2008 “Important role of the LKB1–AMPK pathway in suppressing tumorigenesis in PTEN-deficient mice”
20. Nagashima 2002 – pathway to cytoskeleton
21. GPCR receptor – cross membrane receptors for oestrogen (ER = nuclear receptor) and for endothelin etc. IUPHAR database see refs Prossnitz, Grant 2007
22. Ouyang 2010 metformin inhibits AMPD therefore the AMP:ATP increases activating AMPK
23. Suzuki 2007 “activation of AMPK increases PPAR_gene expression in muscle cells (19).” “Activated _2AMPK phosphorylates acetyl coenzyme A (CoA) carboxylase (ACC), resulting in the inhibition of its activity and reduced formation of malonyl-CoA. The latter effect in turn results in the activation of carnitine palmitoyl transferase 1, a step required for the stimulation of fatty acid oxidation in mitochondria (27).”
24. Oikari 2008 “This indicates that, in our tg rats, increased AMPKa levels might be responsible for the reduction of SREBP-1”.
25. Shaw 2005 “GSK-3 also phosphorylates transcription factors that govern cell fate and differentiation, including c-Jun, ß-catenin, GLI, Notch, Snail and sterolregulatory-element-binding transcription factor 1 (SREBP1)”.
26. Zhou 2001 “AMPK activation suppresses SREBP-1. SREBP-1c is an important insulin stimulated transcription factor that is implicated in the pathogenesis of insulin resistance, dyslipidemia, and DM2 (35, 36). Target genes that are induced by SREBP-1 include those that encode lipogenic enzymes, such as FAS and S14.”
27. Okada 2004 “However, activation of the p53 target protein WAF1 is necessary for the G1-like arrest.” “Phosphorylated CDC25C associates with the p53 target molecule 14-3-3s and is exported from the nucleus, resulting in CDK1 inactivation and the establishment of G2 arrest. p53 sustains G2 arrest through another downstream target molecule, WAF1 (REFS 70,71).”
28. Shaw 2009 & Gwinn 2008 direct inhibitory phosporylation of Raptor by AMPK
29. Chan 2004, Wong 2009, Wang 2011 inhibition of eEF2 by AMPK thus protein synthesis
30. Ben Sahra 2008 Direct action on cyclin D1
31. Buzzai 2007 metformin selectively inhibits p53-deficient cell growth and induces autophagy.