Current Trends in Biotechnology and Pharmacy
Vol. 12 (2) 196-205 April 2018, ISSN 0973-8916 (Print), 2230-7303 (Online)

Targeting Cancer cell metabolism via Target of Rapamycin

Ankita Awasthi[1], Vikrant Nain[1], Himanshi Singh[1], Pavan Kumar[1], Rekha Puria[1*],
1. School of Biotechnology, Gautam Buddha University, Greater NOIDA,
Gautam Budh Nagar-201312, India
* For Correspondence - rpuria@gbu.ac.in

Abstract

Cancer cells acquire many metabolic rearrangements to provide energy and macromolecules required for continuous growth and proliferation. Warburg suggested that cancerous cells rely only on glycolysis for energy and biosynthesis of macromolecules. It's still unexplainable that how this metabolic switch allows predominance of cancer cell in the hypoxic and metabolically highly active conditions around the cancerous cells. Understanding of signaling particularly, role of Ser/Thr PI3 kinase Target of Rapamycin TOR, "central regulator of growth" in cancer cell metabolism has ignited interest in comprehending the precise mechanism linking cancer cell environment to metabolic rearrangements ensuring cancer proliferation. The focus of present review is to summarize the role of TOR in metabolic rearrangements prevalent in glycolysis and TCA occurring in cancerous cell. The insights from mechanistic of mTOR signaling in cancer cell metabolism have led to identification of several downstream candidates to be explored in anticancer therapeutics. Thus usage of drug directly targeting macromolecular biosynthesis in combination with environment responder, mTOR inhibitor, is more promising in cancer therapeutics.

Introduction

Cell growth is a phenomenon that relies on ability of cell to biosynthesize macromolecules and drive energy. It's an incompletely understood complex phenomenon that involves direct communication between extra and intracellular environment. Any communication gap between the two, because of mechanistic failure at any step either results in cell death or uncontrolled proliferation of cells. Randomly growing cells need to establish their predominance in available variable environmental conditions to promote tumorigenesis. To accommodate these observations, Warburg in 1927 predicted that cancerous cells heavily rely on glycolysis rather than oxidative metabolism. These cells generate lactate from glucose inspite of adequate oxygen supply and utilize glucose for macromolecular synthesis (anabolism). Its established now that aerobic glycolysis, uptake of glutamine and glycine allows cancer cells to produce energy and biosynthesize macromolecule(Teicher *et al.* 2012). A thorough understanding of growth response to diverse environmental cues is mandatory for development of efficient cancer therapeutics.

Of late, mTOR has become a favorite candidate in cancer therapeutics. It regulates cell growth in response to different environmental conditions (energy, stress, hypoxia, growth factors etc.) by regulating processes such as translation, transcription, autophagy etc. It catalyzes number of growth process by participating as component of two Complexes mTORC1 and mTORC2. mTORC1 primarily regulates temporal aspect while mTORC2 regulates spatial aspect of growth(Suzuki and Inoki 2011). Interestingly, mTOR is activated in more than 80% of cancers. Its association with cancers is validated by

presence of several oncogenic mutations in components that act upstream of mTOR as well as in mTOR itself(Murugan *et al.* 2013). mTORC1 facilitates alteration of cell metabolism best suited to promote tumorigenesis. It plays role in almost all process e.g., glycolysis, lipogenesis, nucleotide biosynthesis, protein synthesis etc, required for continuous synthesis of macromolecules in growing cancer and provides an explanation to Warburg effect. Here our focus is to summarize the role of mTOR in metabolic rearrangements in cancer which can be exploited in development of new therapeutic interventions with the ability of targeted therapy.

Role of mTOR in cell growth metabolism : mTORC1primarily controls cell growth by regulating the activity of eIF4 and S6 kinase, the two well established substrates of TOR kinase. These two substrates precisely regulate protein synthesis, ribosome biogenesis, stress response etc. The pathophysiology of cancer is supported by 3 major metabolic pathways i.e., A. Glycolysis, B. Pentose Phosphate Pathway (PPP) and C. Lipogenesis. Largely mTORC1 regulates these pathways by controlling the expression of transcription factors HIF1α and SREBP. Basically it drives glucose uptake and glycolysis through upregulation of HIF1 α , induced under low oxygen concentration. It not only induces the expression of enzymes involved in glycolysis instead also promotes uptake of glucose by regulation of expression of glucose transporters (Pinheiro *et al.* 2010). Moreover, activated mTORC1 induces the expression of lipid and sterol biosynthetic genes by stimulating the activity of SREBP. Interestingly SREBP also increases the expression of G6PD rate limiting enzyme of oxidative branch of PPP in mTORC1 dependent manner (Wang *et al.* 2014). This indirectly helps in nucleotide synthesis. Recently it has also been shown that mTORC1 regulates pyrimidine biosynthesis in an S6K1 dependent manner. Hyperactive mTOR phosphorylates S6K1 which in turn phosphorylates CAD, a multifunctional enzyme that catalyzes the three steps in pyrimidine synthesis. Further the role of ACLY in histone acetylation is mediated by SREBP. This implicates global role of mTORC1 in regulation of expression of various genes by chromatin modification. SREBP also plays crucial role in regulation of genes associated with lipogenesis. mTOR also responds to energy stress via AMPK. Increased AMP/ATP ratio induces AMPK which in turn phosphorylates raptor and suppress mTORC1in response to depleted energy levels (Gwinn *et al.* 2008). An ATP dependent TTT-RUVBL1/L complex remains dissociated, and prevents interaction between Rag and mTORC1 required for lysosomal localization and amino acid based mTORC1 activation(Kim *et al.* 2013). Interestingly, a recent study has demonstrated the role of mTOR in reprogramming cellular metabolism from glycolysis to oxidative phosphorylation upon relocalization onto mitochondria (Lu *et al.* 2015). Basically this can increase sensitivity of cancerous cells to various metabolic inhibitors.

Regulation of Glycolysis by mTOR : Glycolysis provides the much needed energy and macromolecules for tumorigenesis. It's a oxidative process where glucose is broken into two 3C pyruvate through series of steps catalyzed by number of enzymes. mTORC1 increases expression of the primary glucose transporter Glut1 and several of the enzymes involved in catalyzing various steps of glycolytic pathway in HIF1α dependent manner. HIF1α being oxygen sensitive, gets rapidly degraded under normal oxygen concentration. Nevertheless, hyperactive TORC1 with increased translation downstream of 4E-BP eIF-4E branch ensures accretion of HIF1α (Ruggero *et al.* 2004). Though most of the enzymes of glycolysis are associated with tumor growth, however role of each in cancer is not well defined.

Hexokinase : It catalyzes the first and rate limiting step of glycolytic pathway i.e., conversion of glucose to to glucose 6-phosphate. Of four mammalian isozymes, HK2 is often hyperactivated in malignant tumors. Its localization onto mitochondrial membrane ensures its ability to couple ATP synthesis to Glucose phosphorylation.

glucose 6-phosphate thus generated is utilized in continuing with glycolysis. This also participates in nucleotide synthesis by contributing in pentose phosphate pathway which is utilized for synthesis of ribose sugars (Kumar *et al.* 1996). Activity of HK2 is regulated by miR125a/b and miR143. Interestingly, mTOR regulates the expression of miR143 which means hyperactivation of mTOR may promote expression of HK2 via downregulation of miR143(Grabiner *et al.* 2014).

Glucose 6 phosphate isomerase (GPI) : GPI or phosphor glucose isomerase catalyzes the reversible isomerization of glucose 6-phosphate to fructose 6-phosphate in glycolysis. GPI is released by tumor cells. It act as autocrine motility factor, induces mitogenic, motogenic and differentiation functions implicated in various aspects of tumor progression and metastasis(Bao *et al.* 2014). It plays crucial role in synthesis of PAP and glycerolipid. Loss of function mutants of GPI results in accumulation of glucose 6-phosphate. It has been observed that an increased concentration of G6P acts as inducer of mTOR signaling in cardiomiocytes. Further GPI deficiency results in increase in PA levels, which has an established role in mTOR signaling(You *et al.* 2014).

Phosphofructokinase : Phosphofructokinase catalyzes rate limiting phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate by using ATP as energy source. It is regulated allosterically by 2,3-diphosphoglycerate. Very little information is available about its direct role either in tumorigenesis or mTOR mediated cancer pathology(Yi *et al.* 2012).

Aldolase : Six carbon sugars are cleaved by an aldolase to produce two 3 carbon triose phosphate glyceraldehyde 3-phosphate and dihydroxy-acetone phosphate. Disruption of Aldolase inhibits cell proliferation by 90% in Ras transformed NIH-3T3 cells. However, this inhibition is neither linked to glycolytic flux nor to levels of intracellular ATP rather probably is outcome of disruption of actin-cytoskeleton dynamics(Lew and Tolan 2012). Since mTORC2 plays role in actin cytoskeleton dynamics, the possible regulatory role of mTORC2 cannot be ignored.

Triose phosphate Isomerase : Triosephosphate isomerase reversibly catalyzes the conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate. No direct correlation of activity of this enzyme with hyperactive mTOR has been reported till date. However, in study it was proposed that upregulation of TPI could possibly retrieve tumor cells from a resting or dormant fashion back to cell cycle, and thus sensitize tumor cells to chemotherapy (Fonvielle *et al.* 2005).

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) : GAPDH catalyzes the oxidation of glycerate 3-phosphate to 1,3 bisphos phateglycerate resulting in ATP synthesis. Overexpression of GAPDH has been observed in various tumor and malignant cell lines e.g., human prostate cancer, lung cancer etc(Guo *et al.* 2013).GAPDH plays significant role in regulating mTOR activity in response to glucose flux. GAPDH can interact with Rheb. Rheb GTP interaction with mTOR is mandatory for responding to amino acid levels in cell (nutrient sensing). When concentration of glucose is low GAPDH remains associated with Rheb so no free Rheb is available to interact with mTOR. However under conditions of high glucose influx GAPDH is primarily involved in carrying out glycolysis and does not interact with Rheb(Lee *et al.* 2009). Thus free Rheb GTP binds to mTOR and promotes cell growth. Basically GAPDH serves as a connecting link between glycolysis and mTORC1 signalling.

Phosphoglycerate kinase : It catalyzes reversible reaction of conversion of substrate 1,3-Biphosphoglycerate to 3 phosphoglycerate with generation of ATP. Out of two isoforms of PGK, PGK1 and PGK2, PGK1 is regulated by hypoxia inducible factor 1α . Increased PGK1 is found in number of different cancers. Recently, PGK1 was identified as substrate of nuclear $p85^{s6k1}$ in phosphoproteomic screen for identification of targets and function of nuclear $p85^{s6k1}$(Jastrzebski *et al.* 2011).

Phosphoglycerate mutase : It catalyzes the interconversion of glycerate 3-phosphate (3PG) and glycerate 2-phosphate (2PG). Altered expression of mutase is observed in different types of cancer. Overexpression of PGAM1 is associated with 66.7% of hepatocellular Carcinoma, Wherein it provides metabolic advantage to cancer cell proliferation and tumor growth. PGAM1 coordinates glycolysis and anabolic biosynthesis partially by controlling intracellular levels of substrate 3PG and product 2PG. 3PG inhibits 6PGD by directly binding to the active site of 6PGD and competing with its substrate 6PG. Attenuation of PGAM1 results in abnormal accumulation of 3PG, which in turn inhibits 6PGD and consequently leads to the oxidative PPP and anabolism (Peng *et al.* 2016).

Enolase : Enolase also known as phosphor pyruvate hydatase catalyzes the penultimate step of glycolysis by converting 2- phosphoglycerate to phosphoenolpyruvate. Promoter contains hypoxia responsive element. EnoA is upregulated at the mRNA and/or protein level in several tumors including brain, breast etc. In cancer cells, EnoA is overexpressed and localizes on their surface where it acts as a key protein in tumor metastasis, promoting cellular metabolism in anaerobic conditions and driving tumor invasion through plasminogen activation and extracellular matrix degradation No direct link of its regulation via mTORC1 has been observed.

Pyruvate Kinase : PK catalyzes the irreversible phosphoryl group transfer from phosphoenol pyruvate to ADP, yielding pyruvate and ATP. Pyruvate kinase is a tetramer that is allosterically activated by PEP and negatively regulated by ATP. PKM2 isoform is exclusively expressed in embryonic, proliferating and tumor cells and it plays an essential role in tumor metabolism and growth. mTOR acts as a central activator of Warburg effect by inducing PKM2 and other glycolytic enzymes under normoxic conditions.mTOR upregulation of PKM2 expression through HIF1α mediated transcription activation and c-myc heterogenous nuclear ribonucleoprotein (hn RNPs) dependent regulation of PKM2 gene splicing. Disruption of PKM2 suppressed oncogenic mTOR mediated tumorigenesis(Sun *et al.* 2011). PKM2 stimulated glycolysis contributes to the development of tumors caused by hyperactive mTOR and therefore this interaction may be targeted in anticancer therapeutics.

Lactate dehydrogenase : Glucose is preferentially converted into lactic acid through aerobic glycolysis. Lactate dehydrogenase catalyzes formation of lactic acid from pyruvate. It's a tetrameric enzyme composed of two subunits (LDHA and LDHB). The exact mechanism by which mTOR regulates the activity of LDH is unknown. However, recent studies have shown that mTOR positively regulates activity of LDHB and signal transducer and activator of transcription 3 (STAT3). STAT3 is a transcription activator of LDHB, downstream of mTOR(Zha *et al.* 2011)

Pyruvate Dehydrogenase : It's a link between glycolysis and TCA cycle. It catalyzes the conversion of pyruvate to acetyl-CoA via decaroxylation. Activity of PDH is regulated by Pyruvate dehydrogenase kinase (PDK1). The ability of HIF1 α to suppress TCA is primarily because of regulation of PDK1. Thus under hypoxic conditions an activated HIF1α activates PDK1 which suppresses PDH by phosphorylation dephosphorylation of three serine residues (ser264, ser271 and ser203) (Seifert *et al.* 2007; Denko 2008; Shi *et al.* 2011)

TCA : Role of TCA cycle in promotion of cancer is not well illustrated. The opponent of Warburg has always suggested an active role of TCA in energy production in cancerous cells. Truncation of TCA cycles due to various physiological or genetic factors does not lead to its complete suppression. Rather utilization of alternative carbon sources, e.g., glutamine ensures replenishment of TCA steps and ensures generation of energy and macromolecules (DeBerardinis *et al.* 2007; Le *et al.* 2012; Nain *et al.* 2014). Interestingly one of the crucial steps in completion of TCA cycle is utilization of glutamine to produce alfa ketoglutarate and activity of

enzyme GDH which catalyzes this conversion is regulated by TOR complex. Basically TOR plays crucial role in TCA by promoting glutamine anaplerosis by activating glutamate dehydrogenase. TORC1 represses the activity of GDH inhibitor, SIRT4 (mitochondria localized sirtuin) by promoting proteasome mediated destabilization of cAMP-responsive element binding 2 (CREB2) (Cibelli *et al.* 1999; Csibi *et al.* 2013). Glutamine is also considered as positive regulator of mTORC1. It facilitates uptake of leucine and promotes mTORC1 assembly and lysosomal localization. Also explains addiction of cancer cells to glutamine. Role of mTOR complex in facilitating other steps of TCA or ETC, ATP synthase activity are not well illustrated.

Lipogenesis and Pentose Phosphate pathway: Recent studies has unraveled prominent role of mTOR in regulating lipogenesis and Pentose Phosphate pathway, important mechanism by which number of tumor cells meet the unique metabolic demands of proliferating cancerous cells. SREBP, transcription factor facilitates these two processes. Akt(upstream regulator of mTOR) regulates SREBP partially by promoting stability of its processed form through inhibition of glycogen synthase kinase (GSK3). GSK3 targets SREBP for proteasomal degradation by its phosphorylation (Jope *et al.* 2007; Xu *et al.* 2009; Dong *et al.* 2016).

SREBP also stimulates the fatty acid synthesis by regulating ACLY at mRNA level. It promotes utilization of acetyl CoA generated upon degradation of citrate to acetyl CoA and oxaloacetate. This requires activity of enzyme ACLY, which has been reported to be upregulated in different types of cancer (Zaidi *et al.* 2012; Khwairakpam *et al.* 2015). AKT/PI3k pathway is responsible for regulation of phosphorylation and activation of ACLY to meet increased demand of fatty acid synthesis generated by membrane biogenesis of growing cells. ACLY is required for histone acetylation which suggests a potential role for global regulation of chromatin downstream of mTORC1 (Covarrubias *et al.* 2016). Further exposure of cancer cell lines to mTOR kinase inhibitor INK128 has been shown to reduce the expression of acetyl CoA carboxylase and fatty acid synthase along with suppressed lipogenesis (Liu *et al.* 2015; Li *et al.* 2016).

mTORC1, regulates pentose phosphate pathway by regulating the expression of glucose-6-phosphate dehydrogenase via SREBP. G6PD is rate limiting enzyme of oxidative branch of pentose phosphate pathway. Thus mTORC1 promotes the utilization of glucose 6 phosphate for synthesis of ribose sugars to be consumed in nucleotide biosynthesis (Ye *et al.* 2012; Stincone *et al.* 2015). Hyperactivation of TOR regulates pyrimidine synthesis by regulation of phosphorylation of enzyme CAD through its downstream effector S6k1 (Magnuson *et al.* 2012; Ben-Sahra *et al.* 2013). Further, G6PD activity results in synthesis of NADPH. High metabolic activity in tumor cells result in generation of high ROS which can actually damage cells. Thus high activity of PPP results in higher NADPH which can be exploited to reduce glutathione in the protection against ROS(Krüger *et al.* 2011; Chadwick *et al.* 2013)

mTOR Inhibitors in cancer therapeutics **:** Crucial role of mTOR in metabolic rearrangements implicate the role of mTOR inhibitors in control of cancerous growth by sabotaging altered metabolism in cancer cells (Fig1). Rapamycin or its analog rapalogs with better pharmaceutical properties exhibit strong anticancer properties. In combination with FKBp12, rapalogs specifically bind FRB domain of Tor kinase and inhibits its activity. Everloimus and temsirolimus (rapalogs) have been approved by FDA for treatment of RCC (Zhang *et al.* 2013).The restricted activity of rapalogs at prescribed concentrations against mTORC1 has limited their ability to circumvent growth of many different types of cancer. Activation of feedback loops and prosurvival pathways by TORC2 supports the proliferation of cancer cells even in presence of rapamycin/rapalogs. The new class of molecular inhibitors with efficacy against both mTORC1 and mTORC2, posses the ability to globally suppress TOR mediated cell growth activities (Wang *et al.* 2009; Xiong *et al.* 2013).

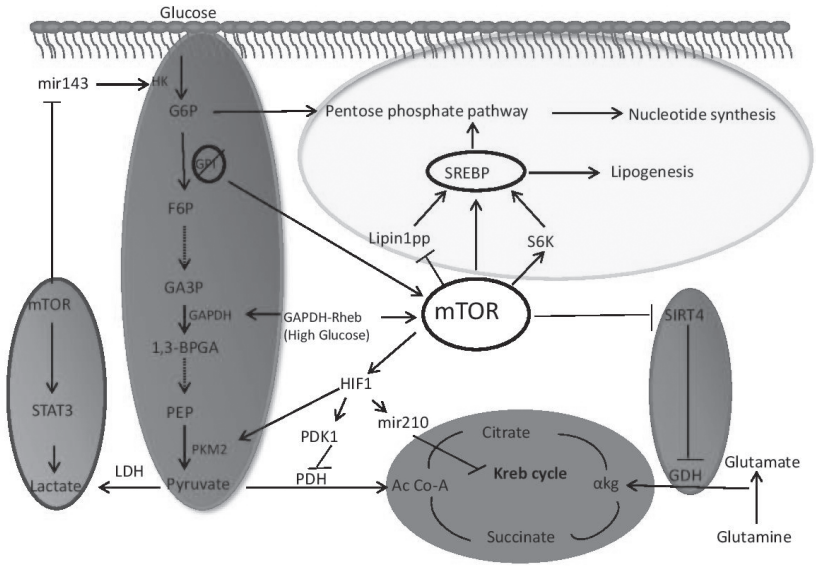

Fig1: mTOR in cancer cell metabolism

Torin1, Torin2 have been shown to possess anticancer properties superior to rapalogs. Nevertheless, high complex cross talk between survival pathways and strong immunosuppression does not rule out the probability of generation of drug resistant mechanism(Thoreen *et al.* 2009; Moorman and Shenk 2010; Hussain *et al.* 2015).

An apparent better alternative is combination therapy e.g. uses of dual inhibitors for PI3k/mTOR. Several clinical trials are going on to search for combination of drugs with high anticancer activity and low toxicity. TKIs are known to reduce tumor cell growth by suppressing glycolysis, reducing lactate production and expression of Glut1, HIF1α and HIF2α (Chiavarina *et al.* 2012; Arreola *et al.* 2014). The use of nontoxic doses of TKIs in combination with known glycolytic inhibitor with low cytotoxicity or with inhibitors specific to components of metabolism which are not directly regulated by mTOR are being tested for their ability to suppress tumorigenesis with high efficacy and low toxicity leading to less side effects.

The advent of various genetic engineering approaches has made it feasible to specifically manipulate the sequence and structure of desired genes at high frequency. The expression of mTOR in cancerous cells can be curtailed by designing and targeting regulatory RNA (miRNA, siRNA) or engineered nucleases (ZFN, TALENS or CRISPR-CAS9) designed against mTOR. (Puria *et al* 2012). Nonetheless genetically modified bacterial strains with ability to recognize and grow precisely at tumor site, are future devices for delivery of gene expression manipulating tools (Regulatory RNA or engineered nucleases) in cancer cells.

Concluding remarks : Reprograming of bioenergetics of cancer cells from glycolysis to oxidative phosphorylation hold promise of efficient cancer therapy. Reduced pyruvate flux into mitochondria oxidative phosphorylation enables cancer cells to avoid ROS generation from oxidative phosphorylation and improvised survival even during metastasis. An induced glycolysis acts as a constant source of energy for proliferating cancer cells. Further expression of alternative routes ensures supply of macromolecules to growing cells. mTOR pathway plays vital role in altered cancer cell metabolism. mTOR regulates the expression of PFK2M and hexokinase largely

responsible for activated uptake and metabolism of glucose. Further mTOR responds to glutamine levels, a source of intermediate for TCA cycle. In addition mTOR mediated expression of miRNA 143 has been shown to promote glucose metabolism in human lung cancer. Actually the understanding of role of mTOR signalling in cancer cell metabolism has expanded the avenues for targeted therapy.Though drugs specific to mTOR are known, an enhanced cytotoxicity has always been a concern. Further it is evident that single factor cannot be responsible for drastic metabolic switch acquired by these cells and cancer cells growing at different regions (periphery, deep inside or during metastasis) faces differential nutrient environment. As mTOR is a sensor of extracellular and intracellular cues, its response should vary under such different conditions. We believe that an understanding of mTOR signalling under varied conditions prevalent during the progression of cancer ultimately resulting in metabolic rearrangements will actually lead to identification of targets with less cytotoxicity and better efficacy.

Acknowledgment
Department of Biotechnology, Ministry of Science &Technology, India to RP is duly acknowledged. Compliance with Ethical Standards.

Conflict of Interest Authors declare no conflict of interest.

References

1. Arreola, A., C. L. Cowey, J. L. Coloff, J. C. Rathmell, and W. K. Rathmell, 2014 HIF1a and HIF2a exert distinct nutrient preferences in renal cells. PLoS One 9: 5.
2. Bao, Q., H. Niess, R. Djafarzadeh, Y. Zhao, B. Schwarz *et al.*, 2014 Recombinant TIMP-1-GPI inhibits growth of fibrosarcoma and enhances tumor sensitivity to doxorubicin. Target. Oncol. 9: 251–261.
3. Ben-Sahra, I., J. J. Howell, J. M. Asara, and B. D. Manning, 2013 Stimulation of de Novo Pyrimidine Synthesis by Growth Signaling Through mTOR and S6K1. Science 339: 1323–1328.
4. Chadwick, A. L., A. Howell, F. Sotgia, and M. P. Lisanti, 2013 Carbonic anhydrase 9 (CA9) and redox signaling in cancer-associated fibroblasts: Therapeutic implications. Cell Cycle 12: 2534.
5. Chiavarina, B., U. E. Martinez-Outschoorn, D. Whitaker-Menezes, A. Howell, H. B. Tanowitz et al., 2012 Metabolic reprogramming and two-compartment tumor metabolism: Opposing role(s) of HIF1a and HIF2a in tumor-associated fibroblasts and human breast cancer cells. Cell Cycle 11: 3280–3289.
6. Cibelli, G., S. Schoch, and G. Thiel, 1999 Nuclear targeting of cAMP response element binding protein 2 (CREB2). Eur. J. Cell Biol. 78:.
7. Covarrubias, A. J., H. I. Aksoylar, J. Yu, N. W. Snyder, A. J. Worth *et al.*, 2016 Akt-mTORC1 signaling regulates Acly to integrate metabolic input to control of macrophage activation. Elife 5:.
8. Csibi, A., S. M. Fendt, C. Li, G. Poulogiannis, A. Y. Choo *et al.*, 2013 The mTORC1 pathway stimulates glutamine metabolism and cell proliferation by repressing SIRT4. Cell 153: 840–854.
9. DeBerardinis, R. J., A. Mancuso, E. Daikhin, I. Nissim, M. Yudkoff *et al.*, 2007 Beyond aerobic glycolysis: Transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc. Natl. Acad. Sci. 104: 19345–19350.
10. Denko, N. C., 2008 Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat. Rev. Cancer 8: 705–713.
11. Dong, Q., F. Giorgianni, S. Beranova-Giorgianni, X. Deng, R. N. O'Meally *et al.*, 2016 Glycogen synthase kinase-3-mediated phosphorylation of serine 73 targets sterol response element binding protein-1c (SREBP-1c) for proteasomal degradation. Biosci. Rep. 36: e00284–e00284.

12. Fonvielle, M., S. Mariano, and M. Therisod, 2005 New inhibitors of rabbit muscle triose-phosphate isomerase. Bioorg. Med. Chem. Lett. 15: 2906–2909.

13. Grabiner, B. C., V. Nardi, K. Birsoy, R. Possemato, K. Shen *et al.*, 2014 A diverse array of cancer-associated MTOR mutations are hyperactivating and can predict rapamycin sensitivity. Cancer Discov. 4: 554–563.

14. Guo, C., S. Liu, and M. Z. Sun, 2013 Novel insight into the role of GAPDH playing in tumor. Clin. Transl. Oncol. 15: 167–172.

15. Gwinn, D. M., D. B. Shackelford, D. F. Egan, M. M. Mihaylova, A. Mery *et al.*, 2008 AMPK Phosphorylation of Raptor Mediates a Metabolic Checkpoint. Mol. Cell 30: 214–226.

16. Hussain, A. R., M. Al-Romaizan, M. Ahmed, S. Thangavel, F. Al-Dayel *et al.*, 2015 Dual Targeting of mTOR Activity with Torin2 Potentiates Anticancer Effects of Cisplatin in Epithelial Ovarian Cancer. Mol. Med. 21: 466–478.

17. Jastrzebski, K., K. M. Hannan, C. M. House, S. S. C. Hung, R. B. Pearson *et al.*, 2011 A phospho-proteomic screen identifies novel S6K1 and mTORC1 substrates revealing additional complexity in the signaling network regulating cell growth. Cell. Signal. 23: 1338–1347.

18. Jope, R. S., C. J. Yuskaitis, and E. Beurel, 2007 Glycogen synthase kinase-3 (GSK3): Inflammation, diseases, and therapeutics. Neurochem. Res. 32: 577–595.

19. Khwairakpam, A. D., M. S. Shyamananda, B. L. Sailo, S. R. Rathnakaram, G. Padmavathi *et al.*, 2015 ATP Citrate Lyase (ACLY): A Promising Target for Cancer Prevention and Treatment. Curr Drug Targets 16: 156–163.

20. Kim, S. G., G. R. Hoffman, G. Poulogiannis, G. R. Buel, Y. J. Jang *et al.*, 2013 Metabolic Stress Controls mTORC1 Lysosomal Localization and Dimerization by Regulating the TTT-RUVBL1/2 Complex. Mol. Cell 49: 172–185.

21. Krüger, A., N.-M. Grüning, M. M. C. Wamelink, M. Kerick, A. Kirpy *et al.*, 2011 The Pentose Phosphate Pathway Is a Metabolic Redox Sensor and Regulates Transcription During the Antioxidant Response. Antioxid. Redox Signal. 15: 311–324.

22. Kumar, A., A. S. Goel, T. M. Hill, S. D. Mikolajczyk, L. S. Millar *et al.*, 1996 Expression of human glandular kallikrein, hK2, in mammalian cells. Cancer Res. 56: 5397–5402.

23. Le, A., A. N. Lane, M. Hamaker, S. Bose, A. Gouw *et al.*, 2012 Glucose-independent glutamine metabolism via TCA cycling for proliferation and survival in b cells. Cell Metab. 15: 110–121.

24. Lee, M. N., S. H. Ha, J. Kim, A. Koh, C. S. Lee *et al.*, 2009 Glycolytic flux signals to mTOR through glyceraldehyde-3-phosphate dehydrogenase-mediated regulation of Rheb. Mol. Cell. Biol. 29: 3991–4001.

25. Lew, C. R., and D. R. Tolan, 2012 Targeting of several glycolytic enzymes using RNA interference reveals aldolase affects cancer cell proliferation through a non-glycolytic mechanism. J. Biol. Chem. 287: 42554–42563.

26. Li, S., Y.-T. Oh, P. Yue, F. R. Khuri, and S.-Y. Sun, 2016 Inhibition of mTOR complex 2 induces GSK3/FBXW7-dependent degradation of sterol regulatory element-binding protein 1 (SREBP1) and suppresses lipogenesis in cancer cells. Oncogene 35: 642–650.

27. Liu, D., Y. Xiao, B. S. Evans, and F. Zhang, 2015 Negative feedback regulation of fatty

acid production based on a malonyl-CoA sensor-actuator. ACS Synth. Biol. 4: 132–140.

28. Lu, C. L., L. Qin, H. C. Liu, D. Candas, M. Fan et al., 2015 Tumor cells switch to mitochondrial oxidative phosphorylation under radiation via mTOR-mediated hexokinase II inhibition - A Warburg-reversing effect. PLoS One 10: 3.

29. Magnuson, B., B. Ekim, and D. C. Fingar, 2012 Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. Biochem. J. 441: 1–21.

30. Moorman, N. J., and T. Shenk, 2010 Rapamycin-Resistant mTORC1 Kinase Activity Is Required for Herpesvirus Replication. J. Virol. 84: 5260–5269.

31. Murugan, A. K., A. Alzahrani, and M. Xing, 2013 Mutations in critical domains confer the human mTOR gene strong tumorigenicity. J. Biol. Chem. 288: 6511–6521.

32. Nain, V., R. Buddham, R. Puria, and S. Sahi, 2014 Role of TCA cycle truncation in cancer cell energetics. Current Trends in Biotechnology and Pharmacy 8: 428-438.

33. Peng, X. C., F. M. Gong, Y. Chen, M. Qiu, K. Cheng *et al.*, 2016 Proteomics identification of PGAM1 as a potential therapeutic target for urothelial bladder cancer. J. Proteomics 132: 85–92.

34. Pinheiro, C. H. da J., L. R. Silveira, R. T. Nachbar, K. F. Vitzel, and R. Curi, 2010 Regulation of glycolysis and expression of glucose metabolism-related genes by reactive oxygen species in contracting skeletal muscle cells. Free Radic. Biol. Med. 48: 953–960.

35. Ruggero, D., L. Montanaro, L. Ma, W. Xu, P. Londei *et al.*, 2004 The translation factor eIF-4E promotes tumor formation and cooperates with c-Myc in lymphomagenesis. Nat. Med. 10: 484–486.

36. Seifert, F., E. Ciszak, L. Korotchkina, R. Golbik, M. Spinka *et al.*, 2007 Phosphorylation of serine 264 impedes active site accessibility in the E1 component of the human pyruvate dehydrogenase multienzyme complex. Biochemistry 46: 6277–6287.

37. Shi, LZ., R. Wang, G. Huang, P. Vogel, G. Neale et al., 2011 HIF1a–dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J. Exp. Med. 208: 1367–1376.

38. Stincone, A., A. Prigione, T. Cramer, M. M. C. Wamelink, K. Campbell *et al.*, 2015 The return of metabolism: Biochemistry and physiology of the pentose phosphate pathway. Biol. Rev. 90: 927–963.

39. Sun, Q., X. Chen, J. Ma, H. Peng, F. Wang *et al.*, 2011 Mammalian target of rapamycin up-regulation of pyruvate kinase isoenzyme type M2 is critical for aerobic glycolysis and tumor growth. Proc. Natl. Acad. Sci. 108: 4129–4134.

40. Suzuki, T., and K. Inoki, 2011 Spatial regulation of the mTORC1 system in amino acids sensing pathway. Acta Biochim. Biophys. Sin. (Shanghai). 43: 671–679.

41. Teicher, B. A., W. M. Linehan, and L. J. Helman, 2012 Targeting cancer metabolism. Clin. Cancer Res. 18: 5537–5545.

42. Thoreen, C. C., S. A. Kang, J. W. Chang, Q. Liu, J. Zhang *et al.*, 2009 An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. J. Biol. Chem. 284: 8023–8032.

43. Wang, T., U. Lao, and B. A. Edgar, 2009 TOR-mediated autophagy regulates cell death in Drosophila neurodegenerative disease. J. Cell Biol. 186: 703–711.

44. Wang, Y. P., L. S. Zhou, Y. Z. Zhao, S. W. Wang, L. L. Chen *et al.*, 2014 Regulation of

G6PD acetylation by SIRT2 and KAT9 modulates NADPH homeostasis and cell survival during oxidative stress. EMBO J. 33: 1304–1320.

45. Xiong, Y., M. McCormack, L. Li, Q. Hall, C. Xiang *et al.*, 2013 Glucose–TOR signalling reprograms the transcriptome and activates meristems. Nature 496: 181–186.

46. Xu, C., N. G. Kim, and B. M. Gumbiner, 2009 Regulation of protein stability by GSK3 mediated phosphorylation. Cell Cycle 8: 4032–4039.

47. Ye, J., A. Mancuso, X. Tong, P. S. Ward, J. Fan *et al.*, 2012 Pyruvate kinase M2 promotes de novo serine synthesis to sustain mTORC1 activity and cell proliferation. Proc. Natl. Acad. Sci. 109: 6904–6909.

48. Yi, W., P. M. Clark, D. E. Mason, M. C. Keenan, C. Hill *et al.*, 2012 Phosphofructokinase 1 Glycosylation Regulates Cell Growth and Metabolism. Science (80-.). 337: 975–980.

49. You, J.-S., H. C. Lincoln, C.-R. Kim, J. W. Frey, C. a Goodman *et al.*, 2014 The role of diacylglycerol kinase æ and phosphatidic acid in the mechanical activation of mammalian target of rapamycin (mTOR) signaling and skeletal muscle hypertrophy. J. Biol. Chem. 289: 1551–63.

50. Zaidi, N., I. Royaux, J. V. Swinnen, and K. Smans, 2012 ATP citrate lyase knockdown induces growth arrest and apoptosis through different cell- and environment-dependent mechanisms. Mol. Cancer Ther. 11: 1925–35.

51. Zha, X., F. Wang, Y. Wang, S. He, Y. Jing *et al.*, 2011 Lactate dehydrogenase B is critical for hyperactive mTOR-mediated tumorigenesis. Cancer Res. 71: 13–18.

52. Zhang, H., D. Berel, Y. Wang, P. Li, N. A. Bhowmick *et al.*, 2013 A Comparison of Ku0063794, a Dual mTORC1 and mTORC2 Inhibitor, and Temsirolimus in Preclinical Renal Cell Carcinoma Models. PLoS One 8:.

53. Puria, R., Sahi. S, Nain. V, 2012 HER2+ breast cancer therapy: by CPP-ZFN mediated targeting of mTOR? Technol Cancer Res Treat. 11: 175-80.