Oncogenic kinases and perturbations in protein synthesis machinery and energetics in neoplasia

Oro Uchenunu[1,2], **Michael Pollak**[1,2,3], **Ivan Topisirovic**[1,2,3,4], **Laura Hulea**[1,3*]

Affiliations: [1]Lady Davis Institute, SMBD JGH, McGill University, [2]Experimental Medicine [3]Gerald Bronfman Department of Oncology, and [4]Biochemistry Department, McGill University, Montreal, Quebec H3T 1E2, Canada

***Correspondence:** laura.hulea@mail.mcgill.ca **(L.H.)**

Key words: Protein synthesis, metabolism, cancer, MTOR, oncogenic kinases

Abstract

Notwithstanding that metabolic perturbations and dysregulated protein synthesis are salient features of cancer, the mechanism underlying coordination of cellular energy balance with mRNA translation (which is the most energy consuming process in the cell) is poorly understood. In this review, we focus on recently emerging insights in the molecular underpinnings of the cross-talk between oncogenic kinases, translational apparatus and cellular energy metabolism. In particular, we focus on the central signaling nodes that regulate these processes (e.g. the mechanistic/mammalian target of rapamycin MTOR) and the potential implications of these findings on improving the anti-neoplastic efficacy of oncogenic kinase inhibitors.

1. Introduction

Protein synthesis is a complex process involving the interaction of ribosomes, mRNAs, tRNAs, and auxiliary proteins known as translation factors (Hershey, et al. 2012). Protein synthesis must be tightly regulated as it affects crucial cellular processes (e.g. proliferation, growth, differentiation and development) (Hershey et al. 2012). Dysregulated mRNA translation is implicated in most hallmarks of cancer including aberrant cell proliferation, survival, angiogenesis and cellular energetics (Hanahan and Weinberg 2011; Johnson, et al. 1976; Kevil, et al. 1996; Larsson, et al. 2007; Larsson, et al. 2006; Topisirovic and Sonenberg 2011). The observation that protein synthesis is altered in malignant cells is not recent. Neoplastic cells were shown to have enlarged and abnormally shaped nucleoli, which are ribosome-producing factories, over a century ago (G. 1896). A positive correlation has been observed between cancer cell proliferation and the rate of protein synthesis (Johnson et al. 1976). Moreover, the function and/or the expression of several components of the translation machinery is perturbed in cancer cells (Ruggero 2013). Oncogene activation and the inactivation of tumour suppressors, which drive the development of cancer, converge on the translation machinery (Ruggero 2013). Dysregulation of the components of the translational machinery results in translational reprogramming that favours the development of drug resistance, angiogenesis, survival, proliferation, and metastasis. For instance, high levels of eukaryotic translation initiator factor 4E (**EIF4E***) have been linked to increased cell cycle progression, neoplastic growth, and chemoresistance (Byrnes, et al. 2007; Larsson et al. 2007).

mRNA translation plays a central role in the regulation of gene expression, leading to immediate changes in protein levels (Sonenberg and Hinnebusch 2009), which are required for adaptation to stress (Guan, et al. 2017; Spriggs, et al. 2010). The importance of gene expression

* Proteins written in bold are represented in the figures.

66 regulation at the translational level is evident as steady-state mRNA levels are thought to have
67 low

concordance with the proteome (Schwanhausser, et al. 2011), although the scope of this phenomenon is still being discussed (Li, et al. 2014b). mRNA translation is one of the most energy demanding cellular processes, requiring ~20%–30% of the total ATP (Buttgereit and Brand 1995; Rolfe and Brown 1997). Thus, in order to sustain elevated protein synthesis required for neoplastic growth, malignant cells must adjust their energy metabolism. MTOR is a key regulator of translation (Sonenberg and Hinnebusch 2009). AMP-activated protein kinase (**AMPK**) acts as an intracellular energy sensor and is activated when nutrients or oxygen are in short supply and the ratio of cellular AMP to ATP is elevated (Kahn, et al. 2005; Shaw 2009). Activated **AMPK** results in the downregulation of protein synthesis, which is accompanied by reduced cell growth and proliferation via the **MTORC1** (mechanistic/mammalian target of rapamycin complex 1) signaling pathway (Shaw, et al. 2004a). Consequently, the **AMPK/MTORC1** signaling pathway links cellular energy status to mRNA translation rates.

It was discovered in the 1920s that cancer cells reprogram their metabolism and reduce glucose to lactate even in the presence of oxygen (Warburg 1925). Tumor cells exhibit elevated glucose uptake as well as lactate production when compared to normal tissues in the presence of oxygen (Warburg 1956). This metabolic reprogramming is referred to as the Warburg effect or "aerobic glycolysis" (DeBerardinis, et al. 2008; Hsu and Sabatini 2008). Although the conversion of glucose to lactate through glycolysis produces far less ATP per glucose molecule than glucose catabolism through oxidative phosphorylation to carbon dioxide and water, during glycolysis ATP is produced at a faster rate, and this may be important to fuel the rapid proliferation of cancer cells (Locasale and Cantley 2011; Shestov, et al. 2014; Vander Heiden, et al. 2009). Thus, increasing glucose uptake and glycolytic flux represents a strategy to quickly generate ATP (Pfeiffer, et al. 2001). Importantly, glycolysis also fuels neoplastic growth through

providing intermediates required for the biosynthesis of lipids, nucleotides, NADPH, and amino acids (Lunt and Vander Heiden 2011). Furthermore, the lactic acid produced as the end product of aerobic glycolysis has been found to favor cancel cell invasion (Smallbone, et al. 2005), used as an alternate tricarboxylic acid cycle (TCA) carbon source (Faubert, et al. 2017), and may interfere with local anti-cancer immune responses (Choi, et al. 2013). The consumption of large amounts of glucose by cancer cells may also suppress the immune response by reducing the glucose concentration in the tumor microenvironment and depriving immune effector cells of glucose (Chang, et al. 2015; Ho, et al. 2015). Moreover, alterations in the tumor microenvironment (such as blood flow, oxygen and nutrient supply) *in vivo* can also contribute to metabolic plasticity (Dang, et al. 2011; Hsu and Sabatini 2008; Jessani, et al. 2004).

In this review, we highlight recent findings related to the role of cancer-relevant signaling pathways in coordinating protein synthesis and metabolic activities in the cell. Furthermore, we speculate on the potential implication of these findings in improving the efficacy of current therapies and in developing future cancer therapeutics.

2. PI3K/AKT – mechanisms of activation and regulation of metabolic functions

The phosphatidylinositol-4,5-biphosphate 3-kinase (**PI3K**)/**AKT**/**MTOR** signaling pathway regulates many essential processes including cell growth, mRNA translation, proliferation, survival, apoptosis, and metabolism (Kauffmann-Zeh, et al. 1997; Laplante and Sabatini 2009b; Yao and Cooper 1995). Aberrant signaling via this pathway has been implicated in pathological conditions including diabetes and cancer, whereby its hyperactivation in general is tumor-promoting (Laplante and Sabatini 2012; Porta, et al. 2014).

The **PI3K/AKT** signaling cascade is activated when receptor tyrosine kinases (**RTKs**) such as insulin receptors are bound by their ligands, including insulin and/or growth factors (Ruggero and Sonenberg 2005) (Figure 1). The extracellular binding of the ligands results in intracellular autophosphorylation of tyrosine residues on the receptors (Lemmon and Schlessinger 2010; Schlessinger 2002). The phosphorylated tyrosine residues recruit **PI3K** to the membrane (Domchek, et al. 1992). At the membrane, **PI3K** phosphorylates phosphatidyl inositol-4,5-biphosphate (PIP_2) to produce phosphatidyl inositol-3,4,5-triphosphate (PIP_3) (Figure 1) (Cantley 2002). PIP_3 then acts as a second messenger and is responsible for translocating downstream signaling proteins such as **AKT/protein kinase B (PKB)** to the cell membrane where they are phosphorylated and activated by **PDPK1** (3-phosphoinositide dependent protein kinase 1) (Figure 1) (Alessi, et al. 1997; Fresno Vara, et al. 2004). **AKT** is a serine/threonine protein kinase that regulates cell survival, growth, and proliferation (Myers and Cantley 2010; Wan, et al. 2007). **AKT** carries out its functions through various downstream effectors including MTOR (Slomovitz and Coleman 2012). A major negative regulator of **AKT** is **PTEN** (phosphatase and tensin homolog) (Stambolic, et al. 1998), which catalyzes the conversion of PIP_3 to PIP_2 and acts as a tumor suppressor (Figure 1). AKT activity is increased in various cancer types, either due to mutations or amplifications of the *AKT1* gene, or due to the dysregulation of upstream regulators (e.g. **PTEN**) and mitogenic factors (e.g. hormones, growth factors) (Cheng, et al. 2005; Malanga, et al. 2008).

The **PI3K/AKT** pathway has been implicated in glucose metabolism and lipid synthesis (Elstrom, et al. 2004; Whiteman, et al. 2002). Specifically, **AKT** has been shown to mediate the translocation of glucose transporter SLC2A4 (solute carrier family 2 member 4; GLUT4) to the plasma membrane (Kohn, et al. 1996) and stimulate glycolysis through the phosphorylation and

activation of 6-phosphofructo-2-kinase (PFK2) (Deprez, et al. 1997). It also indirectly stimulates glycogen synthase to produce glycogen through the phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3) alpha and beta isoforms (Cross, et al. 1995). In addition, **AKT** inhibits gluconeogenesis by phosphorylating and inhibiting forkhead box O1 (FOXO1) transcription factor (Accili and Arden 2004). **AKT** has been implicated in activating ATP-citrate lyase (ACLY), an enzyme involved in fatty acid synthesis in adipocytes (Berwick, et al. 2002). In addition to these findings highlighting the role of **PI3K/AKT** signaling pathway in glucose and lipid metabolism, the **PI3K/AKT** pathway affects cellular metabolic programs via the MTOR pathway (discussed in more detail below).

3. MTOR

MTOR is a conserved serine/threonine kinase that is part of the phosphoinositide kinase-related family which stimulates anabolic processes in the cell, including lipid and protein synthesis (Laplante and Sabatini 2009a; Wang and Proud 2006). It integrates extracellular and intracellular signals emanating from environmental cues, nutrient availability and cellular energetic status (Liu, et al. 2009; Zhou and Huang 2011). In turn, it regulates cell growth, proliferation, protein synthesis, survival, autophagy, and energy metabolism (Shimobayashi and Hall 2014). MTOR is the catalytic subunit of 2 functionally and structurally distinct multiprotein complexes: **MTORC1** and **MTORC2**. One of the main modulators of **MTORC1** activity is the **PI3K/AKT** pathway (Hay and Sonenberg 2004).

3.1. Regulation of MTORC1 activity

Upon activation of the pathway, **AKT** phosphorylates TSC complex subunit 2 (**TSC2**), which heterotrimerise with TSC complex subunit 1 (**TSC1**) and TBC1 domain family member 7 (**TBC1D7**) (Zech, et al. 2016) (Figure 1). Phosphorylation of **TSC2** leads to the inhibition of the **TSC** complex. Since the **TSC** is a GTPase Activating Protein (GAP) complex for the Ras homolog, enriched in brain (**RHEB**), the inhibition of **TSC2** results in increased **RHEB**:GTP levels (Long, et al. 2005; Sancak, et al. 2007). GTP-bound **RHEB** activates **MTORC1** (Figure 1) (Long et al. 2005; Sancak et al. 2007).

In addition to growth factors, hormones and cytokines, which regulate MTOR activity chiefly via **AKT**, the **TSC** integrates other upstream signals to regulate via **MTORC1**. High AMP:ATP and/or ADP:ATP ratios lead to AMP and/or ADP binding to **AMPK** (Figure 1). This leads to its activation, which is further potentiated by serine/threonine kinase 11 (STK11) (Shaw, et al. 2004b). **AMPK** phosphorylates **TSC2,** leading to its activation and the suppression of **MTORC1** signaling (Inoki, et al. 2006). **AMPK** can also be activated by glucose deprivation through an AMP/ATP-independent mechanism (Figure 1), which is triggered by a glucose deprivation-induced decrease in fructose-1,6-bisphosphate levels and mediated by aldolase (Zhang, et al. 2017).

Other signal transduction pathways converge on the **TSC** to exert their effects on **MTORC1**. For instance, the **RAS-RAF-MEK-MAPK** signaling pathway, which is activated by growth factors and frequently upregulated in cancer, can phosphorylate **TSC2** directly or indirectly, via ribosomal protein S6 kinase A1 (**RPS6A1**), leading to stimulation of **MTORC1** (Memmott and Dennis 2009; Roux, et al. 2004) (Figure 1). In addition, studies have shown that **DDIT4** (DNA damage inducible transcript 4) downregulates **MTORC1** activity via **TSC2** (Brugarolas, et al. 2004; DeYoung, et al. 2008) (Figure 1). In response to hypoxia, **DDIT4**

mediated the dissociation of inhibitory 14–3–3 from the **TSC2** protein so as to inhibit **MTORC1** activity (DeYoung et al. 2008). Overall, **MTORC1** acts as an integrator of major regulatory inputs in the form of hypoxia, nutrients, energetic stress and growth factors, mostly via **TSC**.

Another important regulator of **MTORC1** activity, the level of amino acids, is discussed in more detail in section 5.2.

3.2. MTORC1 and the regulation of mRNA translation

mRNA translation occurs in 4 sequential steps: initiation, elongation, termination and ribosome recycling (Hershey et al. 2012). It is mainly regulated at the initiation phase which is composed of two rate-limiting steps (Sonenberg and Hinnebusch 2009). This includes (i) the formation of the 43S pre-initiation complex (PIC) and (ii) the assembly of the **EIF4F** complex on the mRNA cap (Sonenberg and Hinnebusch 2009). The initiation phase of mRNA cap-dependent translation involves the assembly of a 43S pre-initiation complex, which comprises the eukaryotic initiation factors (EIFs) EIF1, EIF1A, EIF3 and EIF5, the 40S ribosomal subunit and the ternary complex (TC). Furthermore, the TC comprises the EIF2 (containing alpha-, beta- and gamma-subunits), bound to GTP and $tRNA_i^{Met}$ (Hinnebusch 2014). The **EIF4F** complex contains 3 subunits: **EIF4E** (mRNA cap-binding subunit), **EIF4A** (DEAD box RNA helicase) and **EIF4G** (scaffolding protein) (Figure 2). The 43S PIC binds to the **EIF4F** complex via the interactions between EIF3 of the 43S PIC and EIF4G to create the 48S PIC (Hinnebusch 2014). The 43S PIC scans the 5' untranslated region (UTR) for the AUG start codon (Hinnebusch 2014). This is an ATP-dependent process that requires the helicase activity of **EIF4A** to unwind secondary structures present in the 5'UTR of mRNAs (Rogers, et al. 1999). The recognition of the AUG start codon causes release of EIFs (Hinnebusch 2014). In addition,

the 60S ribosomal subunit joins the 40S subunit to form the 80S ribosome (Hinnebusch 2014). This process is facilitated by EIF5B-GTP hydrolysis (Hinnebusch 2014). Assembly of the 80S ribosome marks the beginning of mRNA translation elongation.

During the elongation phase of mRNA translation, which is mediated by eukaryotic translation elongation factors (EEFs) (Mohr and Sonenberg 2012), the mRNA codons dictate the sequence of specific tRNAs that go through the acylation-peptidyl-exit (APE) sites of ribosomes to form newly synthesized polypeptides (Jan, et al. 2016). EEF1A (a G-protein), when bound to GTP, loads an amino-acyl charged tRNA into the A-site of the ribosome (Mohr and Sonenberg 2012). The bound GTP is hydrolyzed when the anticodon of the incoming tRNA is matched against the corresponding mRNA codon (Agirrezabala and Frank 2009). This process results in the formation of a peptide bond (Mohr and Sonenberg 2012). The activity of EEF1A is regulated by EEF1B [a guanine exchange factor (GEF)] (Mohr and Sonenberg 2012). EEF2 facilitates the translocation of the ribosome along the mRNA molecule (Taylor, et al. 2007), leading to the uncharged tRNA molecule moving to the E-site and the freeing of the A-site (Starosta, et al. 2014). The hydrolysis of another GTP molecule is required to catalyze the translocation of the ribosome (Stark, et al. 2000). Elongation of the newly synthesized polypeptide continues until a stop codon is encountered on the mRNA molecule. For the termination step of mRNA translation, eukaryotic release factors (eRFs) recognize the stop codons, release the newly synthesized polypeptide and detach the 80S ribosome from the mRNA transcript (Dever and Green 2012). ETF1 (eukaryotic translation termination factor 1) mediates the hydrolysis of peptidyl-tRNA (Alkalaeva, et al. 2006), in cooperation with GSPT1 (eRF3) (Alkalaeva et al. 2006). After this step, the mRNA and deacetylated tRNA are released and the ribosome dissociates into its subunits and is recycled (Kiel, et al. 2007).

Malignant cells are characterized by their ability to proliferate uncontrollably, which correlates with their increased protein synthesis. The ability of cells to upregulate protein synthesis in response to increased physiological demands is in part mediated at the level of ribosome biogenesis (van Riggelen, et al. 2010). Similar to protein synthesis, ribosome biogenesis is a complex multifactorial process that requires careful coordination and regulation. The role MTOR plays in regulating ribosome biogenesis has been extensively reviewed (Gentilella, et al. 2015).

MTORC1 acts as a regulator of both translation initiation and elongation processes (Hsieh, et al. 2012; Proud 2013; Thoreen, et al. 2012; Wang, et al. 2001). To date, eukaryotic translation initiation factors 4E binding proteins (**EIF4EBP1-3** in mammals) and ribosomal protein S6 kinases (**RPS6KB1** and **RPS6KB2** in mammals) represent the best understood mediators of the effects of MTOR on protein synthesis (Figure 2). **MTORC1** phosphorylates **EIF4EBPs** (at Thr 37/Thr 46, followed by Thr 70 and finally Ser 65 in human **EIF4EBP1)** (Brunn, et al. 1997; Gingras, et al. 2001). Unphosphorylated **EIF4EBPs** sequester **EIF4E** and prevent its association with **EIF4G** (Figure 2). Upon **EIF4EBPs** phosphorylation, **EIF4E** is released to form the active **EIF4F** complex (Sonenberg and Hinnebusch 2009). In addition, MAPK (mitogen-activated protein kinase) interacting serine/threonine kinases (MKNKs) regulate mRNA translation through the phosphorylation of **EIF4E** residue on Ser209 (Waskiewicz, et al. 1999). MKNK1 and MKNK2 phosphorylate **EIF4E** following MAPK14 and **MAPK1/3** signaling pathways activation in response to cellular stress and mitogens, respectively (Flynn, et al. 1997; Knauf, et al. 2001; Waskiewicz, et al. 1997). The **EIF4F** complex associates with MKNK1 via the carboxyl terminus of **EIF4G** (Pyronnet, et al. 1999). The phosphorylation of **EIF4E** has been shown to affect EIF4E:mRNA cap association rates (Slepenkov, et al. 2006).

This suggests that EIF4E phosphorylation may affect the EIF4F complex assembly and/or binding of EIF4E to the mRNA cap (Scheper, et al. 2002). Indeed, EIF4E phosphorylation increases the oncogenic potential of EIF4E (Topisirovic, et al. 2004; Wendel, et al. 2007) and is required for metastatic spread of the disease by selectively increasing translation of mRNAs encoding pro-survival (MCL1), pro-metastatic proteins [e.g. SNAIl (snail family transcriptional repressor 1), MMPs (matrix metallopeptidases)] and cytokines (Furic, et al. 2010; Robichaud, et al. 2015). Overall, **EIF4F** assembly is required for recruiting mRNAs to the ribosome, which an essential step in initiating cap-dependent mRNA translation (Gingras, et al. 1999; Pause, et al. 1994).

MTORC1 also controls protein synthesis through the phosphorylation and activation of **RPS6KBs** (Roux and Topisirovic 2012). Activated **RPS6KBs** phosphorylate ribosomal protein S6 (**RPS6**; a component of the 40S ribosomal subunit) (Banerjee, et al. 1990), **EIF4B** (an auxiliary factor which stimulates **EIF4A** helicase) (Raught, et al. 2004), and programmed cell death 4 (**PDCD4**; a negative regulator of the **EIF4A** function) (Chauvin, et al. 2014; Dorrello, et al. 2006; Holz, et al. 2005) (Figure 2). Consequently, **RPS6KBs** indirectly increase **EIF4A** function in two ways: by activating its binding partner **EIF4B** and by phosphorylating and targeting for degradation its negative regulator **PDCD4**, to release it from the **PDCD4-EIF4A** complex (Dennis, et al. 2012; Dorrello et al. 2006). In addition to mediating the effects of **MTORC1** on translation initiation, **RPS6KBs** influence translation elongation. **RPS6KBs** phosphorylate and inactivate eukaryotic elongation factor 2 kinase (**EEF2K)**, thereby preventing the phosphorylation and repression of its target **EEF2** on the Thr56 residue. This facilitates translation elongation (Carlberg, et al. 1990; Wang et al. 2001). **EEF2K** can also be directly phosphorylated by MTOR and **AMPK** (Browne, et al. 2004; Browne and Proud 2004). mRNA

translation-related processes which are regulated by different signaling pathways have been recently reviewed [in (Roux and Topisirovic 2018)].

Although the activation of **MTORC1** correlates with increased global protein synthesis, it also leads to qualitative perturbations of the translatome (Meyuhas and Dreazen 2009). **MTORC1** preferentially enhances the translation of a subset of mRNAs bearing a series of 4-14 pyrimidines following the C nucleotide found immediately after the 5' mRNA cap structure (Meyuhas and Dreazen 2009). This motif is referred to as the 5′ terminal oligopyrimidine (5′ TOP) motif. The vast majority of TOP mRNAs encode components of the translational machinery such as ribosomal proteins, **EEF2** and poly (A)-binding proteins (PABPs), and their translation is dramatically suppressed by MTOR inhibitors (Hsieh et al. 2012; Meyuhas and Dreazen 2009). Initially, it was proposed that the **RPS6KBs**/**RPS6** axis mediated the regulatory effects of MTOR on the translation of TOP mRNAs (Jefferies, et al. 1997; Jefferies, et al. 1994). Subsequently, it was however found that there was no difference in the translation of TOP mRNAs when cells deficient in **RPS6KBs** and expressing non-phosphorylable **RPS6** (i.e. **RPS6** knock-in) were compared to wild type cells (Pende, et al. 2004; Ruvinsky, et al. 2005). In addition, although **EIF4EBPs** have been implicated in regulation of TOP mRNA translation (Thoreen et al. 2012), it has been shown that this is likely not the case in response to physiological stimuli (Miloslavski, et al. 2014). Several additional factors recently emerged as potential mediators of **MTORC1** signaling on the translation of TOP mRNA transcripts, such as La ribonucleoprotein domain family member 1 (LARP1) and TIA1/TIAL1 (Fonseca, et al. 2015; Hong, et al. 2017; Philippe, et al. 2018;

295 Tcherkezian, et al. 2014). Furthermore, the context in which translation takes place is known
296 to affect the translation of TOP mRNAs.

297 In addition to TOP mRNAs, other subsets of mRNAs have been shown to be affected by
298 changes in MTOR activity (Gandin, et al. 2016; Larsson, et al. 2012). These mRNAs, commonly
299 referred to as "EIF4E-sensitive", are highly dependent on **EIF4E** levels and/or availability. They
300 mostly have long and highly structured, G/C-rich 5' UTRs and have a high requirement for the
301 helicase activity of **EIF4A**, activity that is potentiated when **EIF4A** is present in the **EIF4F**
302 complex (Koromilas, et al. 1992; Silvera, et al. 2010; Svitkin, et al. 2001) (Figure 2). Some of
303 these mRNAs encode cell cycle regulators such as cyclins (cyclin D1), pro-survival proteins
304 [**BCL2**, **MCL1**, BCL2L1 (BCL2 like 1) and BIRC5], oncogenes (**MYC**, PIM1) and other
305 proteins critical to cell proliferation [ornithine decarboxylase 1(ODC1)] (De Benedetti and Graff
306 2004; Mamane, et al. 2004; Martelli, et al. 2012; Martelli, et al. 2011). The translational
307 regulation of these mRNAs is EIF4EBP-dependent, underlying the major role of **EIF4EBPs** in
308 meditating the effects of MTOR on cell proliferation (Dowling, et al. 2010).

309 A subset of "EIF4E-sensitive" mRNAs harbors very short 5'UTRs, which are enriched in
310 TISU elements (Translation Initiator of Short 5′ UTR; SAASATGGCGGC, in which S is C or
311 G) (Elfakess, et al. 2011). Many of the "EIF4E-sensitive" genes with very short 5'UTR encode
312 proteins involved in mitochondrial activity and biogenesis (discussed in more detail below)
313 (Morita, et al. 2013) (Figure 2) and are less dependent on **EIF4A** activity (Gandin et al. 2016).
314 This differentiates them from mRNAs with long 5' UTRs, encoding pro-proliferative and pro-
315 survival proteins, which are both EIF4E- and EIF4A-sensitive (Gandin et al. 2016). As a
316 consequence, the changes in the translational program induced by the inhibition of **EIF4A** differ

from those induced by **EIF4E** inhibition and lead to a different metabolic and cell fate effect, which will be described in more detail in the following sections (section 7) (Gandin et al. 2016).

3.3. MTOR (MTORC1): master metabolic hub

Malignant cells are characterized by uncontrolled cell proliferation and growth, which are made possible in part through translational and metabolic rewiring (Hanahan and Weinberg 2011). In response to nutrients (glucose and amino acids), energetic requirements and growth factors (Dibble, et al. 2012; Efeyan, et al. 2013; Gwinn, et al. 2008; Inoki, et al. 2003; Zoncu, et al. 2011), **MTORC1** regulates different metabolic processes, such as the biosynthesis of proteins, lipids and nucleotides, and autophagy (Ben-Sahra, et al. 2013; Duvel, et al. 2010; Holz et al. 2005; Kim, et al. 2011).

3.3.1. MTOR regulates glucose and glutamine metabolism

MTORC1 stimulates glycolysis in part through the translational regulation of transcription factors such as **MYC** and hypoxia inducible factor 1A (**HIF1A**) (Duvel et al. 2010; Gordan, et al. 2007). In some cell types, **MTORC1** regulates **HIF1A** translationally via **EIF4EBP1** and **RPS6KB1** (Dodd, et al. 2015) (Figure 2). **MTORC1** also enhances the transcription of *HIF1A* mRNA by phosphorylating **STAT3** (signal transducer and activator of transcription 3), which leads to **HIF1A** protein accumulation during hypoxia (Dodd et al. 2015).

HIF1A stimulates glucose flux and glycolysis through the activation of SLC2A1 (solute carrier family 2 member 1) transporter and of glycolytic proteins such as hexokinase, pyruvate kinase and phosphofructokinase (Keith, et al. 2011; Semenza 2000; Wenger 2000). **MYC** has also been shown to upregulate the transcription of genes involved in glucose metabolism

(Gordan et al. 2007; Stine, et al. 2015). In fact, various proteins involved in glucose metabolism, such as lactate dehydrogenase (LDHA), phosphofructokinase, glucose transporter SLC2A1, hexokinase and PKM2 (pyruvate kinase M2) are both **MYC** and **HIF1A** targets (Kim, et al. 2007; Osthus, et al. 2000; Shim, et al. 1997).

Glutamine is one of the most readily available nonessential amino acids used by malignant cells. It serves as an important source of energy, carbon and nitrogen for various anabolic reactions (Reitzer, et al. 1979; Wise, et al. 2008). Glutamine is the main contributor to the TCA cycle anaplerosis (replenishment of TCA cycle intermediates) (DeBerardinis, et al. 2007), whereby TCA intermediates are used for lipid, nucleotide and amino acid synthesis (Wise and Thompson 2010). Activated **MTORC1** stimulates glutaminolysis, whereby glutamine is converted to glutamate by glutaminase (**GLS**) (Figure 2) (Csibi, et al. 2013). α-ketoglutarate, which is produced from glutamate by glutamate dehydrogenase (**GLUD1**), feeds into the TCA cycle (Csibi et al. 2013). One of the mechanisms by which **MTORC1** promotes glutamine TCA anaplerosis is by indirectly inducing the transcriptional repression of ***SIRT4*** (sirtuin 4), an inhibitor of **GLUD1** activity, leading to **GLUD1** activation (Csibi et al. 2013). This is achieved by MTORC1-mediated degradation of **ATF2** (activating transcription factor 2), which is a transcription factor for ***SIRT4*** (Csibi et al. 2013) (Figure 2). Another mechanism by which **MTORC1** activates TCA anaplerosis and affects glutamine metabolism is by positively regulating **GLS** levels through RPS6KB1-dependent regulation of **MYC** (Csibi, et al. 2014). **RPS6KB1** modulates the phosphorylation of **EIF4B**, which is necessary to the unwinding of the structured 5'UTR of **MYC** by **EIF4A** (Csibi et al. 2014) (Figure 2).

In conclusion, **MTORC1** modulates the uptake and/or metabolism of glucose and glutamine, the two main nutrients fueling cancer cells, through multiple mechanisms and layers of regulation.

3.3.2. MTORC1 in regulating amino acids homeostasis

Amino acids are not only required for protein synthesis but also serve as substrates for a variety of metabolic pathways and are major regulators of **MTORC1** activity (Saxton and Sabatini 2017). In mammals, heterodimeric **RRAG** (Ras related GTP binding) GTPases regulate **MTORC1** signaling in response to amino acid levels (Figure 1) (Kim, et al. 2008; Sancak, et al. 2008). **RRAGs** form heterodimers of **RRAGA** or **RRAGB** in combination with **RRAGC** or **RRAGD**, respectively (Kim et al. 2008; Sancak et al. 2008). **RRAG** heterodimers associate with lysosomal membrane through their interaction with the Ragulator complex. The Ragulator complex, also known as **LAMTOR** (late endosomal/lysosomal adaptor, MAPK and MTOR activator), is comprised of CDKN2C (cyclin-dependent kinase inhibitor 2C), CDKN2A (cyclin-dependent kinase inhibitor 2A), LAMTOR3, LAMTOR4 and LAMTOR5 (Sancak, et al. 2010). **LAMTOR** acts as a GEF towards **RRAGs** (Bar-Peled, et al. 2012; Sancak et al. 2010). The presence of amino acids stimulates **RRAG** heterodimers whereby **RRAGC/D** and **RRAGA/B** are GDP- and GTP-bound, respectively (Sancak et al. 2008) (Figure 1). Active **RRAG** heterodimers recruit **MTORC1** to the lysosomal surface via the interaction between the **RRAGs** and the **MTORC1** subunit RPTOR (regulatory associated protein of MTOR complex 1), where **MTORC1** becomes activated by **RHEB** (Bar-Peled et al. 2012). More recently the mechanistic

insights in the complexity of the control of **MTORC1** activity by amino acids have been unraveled. For instance, the lysosomal v-ATPase interacts and stimulates the GEF activity of the **LAMTOR** complex in response to amino acids (Zoncu et al. 2011). Lysosomal amino acid transporter SLC38A9 has been implicated in interacting with the **RRAG-LAMTOR**-v-ATPase complex, which is necessary for arginine-dependent activation of **MTORC1** (Jung, et al. 2015). In addition, GATOR1 and GATOR2 complexes have been identified as regulators of **MTORC1** signaling through their interaction with the **RRAGs** (Bar-Peled, et al. 2013). The GATOR1 complex, which is composed of DEPDC5 (DEP domain containing 5), NPRL2 (NPR2 like), and NPRL3 (NPR3 like), is a negative regulator of **MTORC1** (Bar-Peled et al. 2013). It acts as a GAP for **RRAGA/B** (Bar-Peled et al. 2013). GATOR2 complex is composed of MIOS (meiosis regulator for oocyte development), WDR24 (WD repeat domain 24), WDR59, SEH1L (SEH1 like nucleoporin), and SEC13 and is a positive regulator of **MTORC1** signaling (Bar-Peled et al. 2013). A newly identified complex called KICSTOR, which is composed of KPTN (kaptin, actin binding protein), ITFG2 (integrin alpha FG-GAP repeat containing 2), C12orf66 (chromosome 12 open reading frame 66), and SZT2 has been shown to interact with GATOR1 on the lysosomal surface (Wolfson, et al. 2017). The complex is important for sensing amino acid or glucose deprivation (Wolfson et al. 2017). In addition, Cellular Arginine Sensor for **MTORC1** (CASTOR1) has been shown to interact with GATOR2 and is necessary for arginine deprivation-induced downregulation of **MTORC1** (Chantranupong, et al. 2016). It is only recently that these different amino acids sensors were discovered and found to modulate the activities of **MTORC1**, opening the possibility for the existence of more amino acid sensors that may modulate **MTORC1** via **RRAGs**.

In addition to being regulated by amino acid availability, **MTORC1** is also involved in stimulating the synthesis of non-essential amino acids. Indirectly, MTOR regulates the synthesis of nonessential amino acids by stimulating glycolysis, TCA cycle and pentose phosphate pathways (Duvel et al. 2010; Yecies and Manning 2011), which provide key metabolites necessary for amino acids synthesis (Duvel et al. 2010; Yecies and Manning 2011) and regulating the translation of mRNAs encoding key enzymes involved in the synthesis of non-essential amino acids (Hulea *et al*., Cell Metabolism, in press; bioRxiv 160879; doi: https://doi.org/10.1101/160879) .

Rapamycin, an allosteric inhibitor of **MTORC1**, causes acute **MTORC1** inhibition by binding to FK506-binding protein (FKBP) which interacts with MTOR and narrows its active site cleft (Harding, et al. 1989; Siekierka, et al. 1989; Yang, et al. 2013) . Rapamycin can also lead to **MTORC2** inhibition after prolonged treatment, in certain cell lines and hepatocytes *in vivo* (Lamming, et al. 2012; Sarbassov, et al. 2006). Rapamycin has been shown to increase the expression of argininosuccinate synthase 1 (ASS1), which stimulates synthesis of arginine (Peng, et al. 2002). Via **MYC**, **MTORC1** indirectly regulates serine hydroxymethyltransferase 2 (SHMT2), involved in glycine synthesis (Nikiforov, et al. 2002). Rapamycin-mediated MTOR inhibition also leads to a decrease in the levels of asparagine, which is linked to a decrease in expression of asparagine synthetase (ASNS) (Peng et al. 2002). Interestingly, it was proposed that asparagine functions as an amino acid exchange factor, regulating the uptake of amino acids (in particular serine, arginine and histidine) (Krall, et al. 2016) and thus stimulating **MTORC1** activity.

These findings add to the increasing amount of evidence highlighting the complexity of regulatory mechanisms whereby **MTORC1** senses amino acids and regulates their utilization and synthesis.

3.3.3. MTOR stimulates lipid synthesis

Rapidly dividing malignant cells require increased synthesis of lipids, which are the main components of plasma and organelle membranes (Menendez and Lupu 2007). **MTORC1** regulates *de novo* lipid synthesis by relaying mitogenic and oncogenic signals to downstream effectors that are important for lipogenesis. Lipid biosynthesis is regulated by the sterol responsive element binding proteins (SREBP1a, 1c and 2), which are activated by low sterol levels. **SREBPs** are transcription factors that regulate the expression of genes involved in the biosynthesis of fatty acids and sterols (Horton, et al. 2002). **MTORC1** activates **SREBPs** in a RPS6KB-dependent manner (Duvel et al. 2010; Li, et al. 2016; Porstmann, et al. 2008) (Figure 2). Consistently, rapamycin downregulates the expression of **SREBP** gene targets including *ACLY, ACACA* (acetyl CoA carboxylase alpha) and *FASN* (fatty acid synthase (Brown, et al. 2007; Porstmann et al. 2008). **MTORC2** inhibition has been shown to reduce the activity of **SREBP1** and the expression of its target genes, such as *ACACA* and *FASN*, which suppresses lipogenesis (Li et al. 2016). The phosphatidic acid phosphatase **LPIN1** has also been implicated in the regulation of lipid metabolism by **MTORC1**. In addition to its role in triglyceride synthesis, by converting phosphatidic acid to diacylglycerol, **LPIN1** is a regulator of **SREBP1** activity (Peterson, et al. 2011). **MTORC1** phosphorylates **LPIN1**, which prevents its translocation to the nucleus and thereby prevents the LIPIN1-dependent suppression of **SREBP** activity (Peterson et al. 2011) (Figure 2). Finally, MTOR can activate **SREBP1** by

phosphorylating CREB regulated transcription coactivator 2 (CRTC2) (Han, et al. 2015), which attenuates CRTC2 inhibitory effects on the processing of **SREBP1** (Han et al. 2015).

In addition to **SREBPs**, **MTORC1** influences lipid metabolism by upregulating the activity of peroxisome proliferator activated receptor gamma (**PPARG**) (Kim and Chen 2004) (Figure 2). Hyperactivation of the **MTORC1** pathway stimulates PPARG-dependent adipogenesis (Zhang, et al. 2009), whilst rapamycin leads to the reduction of both mRNA and protein levels of **PPARG** and the inhibition of adipogenesis (Cho, et al. 2004; Kim and Chen 2004). There is evidence showing that **MTORC1** mediates its effects on the regulation of **PPARG** via **EIF4EBPs** and **RPS6KB1** (Le Bacquer, et al. 2007; Um, et al. 2004). Disruption of **EIF4EBP1** and **EIF4EBP2** in mice led to increased sensitivity to diet-induced obesity driven by increased expression of CCAAT/enhancer-binding proteins (CEBPD, CEBPA) and **PPARG** (Le Bacquer et al. 2007). This was associated with reduced energy expenditure, reduced lipolysis, and greater fatty acid re-esterification in the adipose tissue (Le Bacquer et al. 2007). Furthermore, resistance to insulin in **EIF4EBP1** and **EIF4EBP2** double knockout mice was associated with increased **RPS6KB** activity, which impaired **AKT** signaling in muscle, liver, and adipose tissue. **LPIN1** also plays a role in the regulation of **PPARG**, acting as its transcriptional coactivator (Koh, et al. 2008) (Figure 2). On the basis of these findings, **MTORC1** regulates lipid synthesis chiefly by perturbing activity of **SREBPs** and **PPARG**.

3.3.4. MTOR and the regulation of PPP and nucleotide synthesis

Pentose phosphate pathway (PPP) is required to generate ribose 5-phosphate from glucose and regenerate NADPH via its oxidative arm (Dickens and Williamson 1956; Glaser and Brown 1955; Horecker, et al. 1951). NADPH is an important reducing equivalent

necessary to fuel various metabolic reactions including lipid biosynthesis and plays an important role in protection from oxidative damage (Oudejans, et al. 1983; Winkler, et al. 1986). Ribose 5-phosphate, which is converted to 5′-phosphoribosyl-1'-pyrophosphate, is an essential precursor for nucleotide synthesis (Hove-Jensen 1989). **MTORC1** has been shown to regulate expression of PPP genes partly through **SREBPs** (Figure 2) (Duvel et al. 2010), whilst **PI3K** inhibition has been shown to inhibit the PPP (Wang, et al. 2009).

The **MTORC1/RPS6KB1** signaling axis stimulates *de novo* pyrimidine synthesis via the phosphorylation of glutamine-dependent carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (**CAD**) (Ben-Sahra et al. 2013; Robitaille, et al. 2013) (Figure 2). This enzyme mediates the formation of the pyrimidine ring (Ben-Sahra et al. 2013; Robitaille et al. 2013). In addition, **MTORC1** transcriptionally regulates multiple enzymes involved in purine synthesis via the ATF4-dependent expression of methylenetetrahydrofolate dehydrogenase 2 (**MTHFD2**) (Ben-Sahra, et al. 2016) (Figure 2). **MTHFD2** is an essential enzyme for the mitochondrial tetrahydrofolate cycle, which provides one-carbon units for purine synthesis (Shuvalov, et al. 2017). Finally, in MYC-transformed cells, phosphoribosyl pyrophosphate synthetase 2 (*PRPS2*) mRNA is translationally regulated in a EIF4E-dependent manner, leading to increased nucleotide biosynthesis (Cunningham, et al. 2014).

By regulating nucleotide synthesis, **MTORC1** provides the building blocks for RNA and DNA synthesis, needed for ribosome biogenesis, cellular growth and proliferation.

3.3.5. The role of MTOR in the regulation of mitochondrial biogenesis and activity

Considering that mRNA translation is a highly energy consuming cellular process, it is closely coordinated with cellular energy production (Topisirovic and Sonenberg 2011). To this end, malignant cells must meet the heightened energy requirement caused by elevated energy consumption by the protein synthesis apparatus (Ruggero 2013; Ward and Thompson 2012). It has been reported that **MTORC1** activity is positively correlated with ATP production (Morita et al. 2013). Rapamycin reduces oxygen consumption and ATP synthetic capacity (Schieke, et al. 2006). **MTORC1** regulates energy production in the mitochondria in a EIF4EBP1-dependent manner, by regulating the translation of nuclear-encoded mitochondria-related mRNAs such as components of complex I and V, mitochondrial ribosomal proteins and transcription factor a, mitochondrial (TFAM) (Figure 2) (Morita et al. 2013). The vast majority of these proteins are encoded by mRNAs harboring short 5'UTR/TISU elements (Gandin et al. 2016), and their translation is EIF4E-sensitive, but not affected by **EIF4A** inhibition, as previously discussed (Roux et al. 2004) (Figure 2). Finally, the **MTORC1**/ **EIF4EBP** axis has been shown to regulate mitochondrial dynamics by modulating translation of mitochondrial fission process 1 (MTFP1) (Morita, et al. 2017).

In addition to translational regulation, **MTORC1** regulates the transcription of mitochondrial genes via PPARG coactivator 1 alpha (**PPARGC1A**) (Figure 2) (Cunningham, et al. 2007). The inhibition of **MTORC1** by rapamycin decreased the expression of mitochondrial transcriptional regulators **PPARGC1A**, estrogen related receptor A (ESRRA) and nuclear respiratory factors (NRF), which resulted in reduced mitochondrial gene expression and oxygen consumption (Cunningham et al. 2007). Further analysis identified the transcription factor Yin-Yang 1(**YY1**) as the common target of MTOR and **PPARGC1A** that is required for rapamycin-dependent repression of those genes (Figure 2) (Cunningham et al. 2007).

Future work is required to establish the coordination of MTORC1-dependent translational and transcriptional programs that govern mitochondrial biogenesis and functions. **MTORC2** was also shown to be important for maintaining mitochondria associated ER membrane integrity (Betz, et al. 2013). **MTORC2** deficiency causes increases in mitochondrial membrane potential, ATP production and calcium uptake (Betz et al. 2013).

3.3.6. Nuclear activity of MTOR

In addition to the previously discussed roles of MTOR in regulating translation, it has emerged that MTOR can directly influence the transcription of metabolic genes of prostate cancer cells via its interaction with androgen receptor in the nucleus (Audet-Walsh, et al. 2017). Interestingly, in castration-resistant prostate cancer cells, MTOR transcriptional activity and modulation of metabolic programs occurred even in the absence of androgens (Audet-Walsh et al. 2017). These results bring forward the importance of nuclear MTOR and the need for additional work to uncover its role in this cellular compartment.

3.3.7. Role of MTORC2 in metabolic regulation

MTORC2 is known to regulate cell survival, metabolism, cytoskeletal organization and cell migration (Oh and Jacinto 2011; Populo, et al. 2012; Soukas, et al. 2009). **MTORC2** also regulates metabolic processes such as glycolysis, glutaminolysis, lipogenesis and nucleotide metabolism (Masui, et al. 2014). Abrogation of **MTORC2** in the liver impaired glycolysis and lipogenesis and led to constitutive gluconeogenesis (Hagiwara, et al. 2012). Consequentially, this led to systemic hyperglycemia, hyperinsulinemia, and hypolipidemia (Hagiwara et al. 2012). In addition, **MTORC2** in adipose tissue appears to systemically affect whole-body growth

(Cybulski, et al. 2009). **MTORC2** has been shown to regulate glycolysis and glutaminolysis indirectly by regulating **MYC** levels through FOXO1 and FOXO3 acetylation (Masui, et al. 2013). However, compared to **MTORC1**, the role of **MTORC2** in metabolic regulation is largely understudied.

4. Therapeutic implications of the cross-talk between translatome and metabolome

The hypothesis that drugs can exploit cancer specific metabolic vulnerabilities (Vander Heiden et al. 2009) is attractive. Since protein synthesis, which is the most energy consuming process in the cell, is also dysregulated in cancer, targeting the translational machinery has also been considered to increase the efficacy of anti-cancer treatments (Hagner, et al. 2010).

Since **MTORC1** acts as a pivotal regulator of major metabolic pathways and protein synthesis, targeting **MTORC1** represents an appealing strategy to simultaneously target translational apparatus and cancer energetics. By inhibiting **MTORC1**, rapamycin induces changes in cellular metabolism, including decrease in mitochondrial activity, amino acid biosynthesis, pentose phosphate pathway, and sterol and lipid biosynthesis (Cunningham et al. 2007; Duvel et al. 2010; Peng et al. 2002; Ramanathan and Schreiber 2009; Schieke et al. 2006). At the organismal level, rapamycin treatment result in hyperglycemia, hyperlipidemia, a decrease in glucose-stimulated insulin synthesis and secretion, and weight loss (Fraenkel, et al. 2008). Overall, rapamycin suppresses key metabolic processes by inhibiting **MTORC1**.

Rapamycin and its analogues (rapalogs; Figure 1) are FDA approved for the treatment of renal cell carcinomas, mantle cell lymphomas and pancreatic neuroendocrine tumours (Li, et al. 2014a). However, the efficacy in the clinic is not as good as initially hoped (Fasolo and Sessa 2008). This can been justified in part by the activation of **AKT** via the suppression of the

564 **RPS6KB1-IRS1-PI3K-AKT** regulatory feedback, as well as by rapamycin's inability of to
565 inhibit certain **MTORC1** outputs including phosphorylation of **EIF4EBPs** (Dowling et al. 2010;
566 Faes, et al. 2017). To this end, more efficient means to target MTOR were developed, including
567 active site MTOR inhibitors (asTORi), which target ATP binding pocket of MTOR, and third-
568 generation MTOR inhibitors (RapaLink-1), which combine allosteric and active site inhibition
569 (Benjamin, et al. 2011; Rodrik-Outmezguine, et al. 2016; Roux and Topisirovic 2012) (Figure 1).
570 The new generation of MTOR inhibitors efficiently suppress **EIF4EBP** phosphorylation and
571 reduce **AKT** signaling via inhibition of **MTORC2** (Benjamin et al. 2011). These inhibitors are
572 presently under investigation in clinical trials and are expected to exhibit enhanced efficacy in
573 the clinic as compared to rapalogs.

574 In addition to MTOR inhibitors, the vast majority of oncogenic kinase inhibitors
575 indirectly suppress **MTORC1** and are thus positioned to alter the crosstalk between translational
576 machinery and energy metabolism in neoplasia. Lapatinib is a dual receptor tyrosine kinase
577 inhibitor (TKI) (Figure 1) of epidermal growth factor receptor (EGFR) and human epidermal
578 growth factor receptor 2 (HER2), which is used for treating HER2-positive breast cancer
579 (Geyer, et al. 2006). Lapatinib inhibits the **RAS-RAF-MEK-MAPK** and **MTORC1** signaling
580 pathways (Brady, et al. 2015). Furthermore, lapatinib inhibits glycolysis and reduces
581 mitochondrial membrane potential (Paech, et al. 2017). Vemurafenib is a BRAF V600E
582 serine/threonine kinase inhibitor used in the treatment of advanced melanoma (Young, et al.
583 2012). It downregulates the **RAS-RAF-MEK-MAPK** and **MTORC1** signaling pathways (Zhan,
584 et al. 2015) (Figure 1). Similar to lapatinib, vemurafenib inhibits glycolysis in melanoma cells
585 (Delgado-Goni, et al. 2016). Furthermore, vemurafenib increases oxidative and anaplerotic
586 pyruvate carboxylase (**PC**) mitochondrial metabolism and decreases lipid synthesis (Delgado-

587 Goni et al. 2016). Imatinib is another TKI that suppresses abnormal activation of the
588 **PI3K/AKT/MTORC1** pathway downstream of a constitutively active BCR/ABL kinase present
589 in chronic myelogenous leukemia (Hirao, et al. 2018). Consistent with most kinase inhibitors
590 (KI), imatinib also inhibits glucose uptake and glycolysis (Boren, et al. 2001; Gottschalk, et al.
591 2004). Moreover, its ability to alter metabolic enzyme activities involved in fatty acid and *de*
592 *novo* nucleic acid synthesis demonstrates the mechanism by which it inhibits leukemia cell
593 growth (Boren et al. 2001).

594 While kinase inhibitors suppress MTOR signaling, their inability to impede **EIF4F**
595 complex assembly dramatically reduces their anti-neoplastic efficacy. For example, in
596 BRAF(V600)-mutated melanoma, resistance to anti-BRAF and anti-MEK therapies, can be
597 overturned by altering **EIF4F** complex activity by using **EIF4A** inhibitors (Boussemart, et al.
598 2014). In a model of mammary epithelial cells, resistance to PI3K/MTOR inhibitor BEZ235 was
599 induced by either **MYC** or **EIF4E** amplification (Ilic, et al. 2011). Resistant cells showed
600 elevated 5' cap-dependent protein translation, supporting the importance of **EIF4F** activity in
601 development of resistance to KI (Ilic et al. 2011). In breast cancer xenografts, over-expressing
602 **EIF4E** induces resistance to HER2 and EGFR inhibitors including lapatinib (Zindy, et al. 2011).
603 Similarly, high **EIF4F** and cap-dependent translation levels occur in non-small cell lung cancer
604 cells resistant to EGFR inhibitor erlotinib (Patel, et al. 2013). More generally, a high
605 **EIF4E/EIF4EBP** ratio was shown to dramatically decrease the efficacy of MTOR inhibitors
606 across multiple cancer cell lines and *in vivo* (Alain, et al. 2012a; Alain, et al. 2012b). Overall,
607 these findings underscore the role of translation machinery in determining the efficacy of MTOR
608 targeted therapies and suggest that the inability of such approaches to suppress mRNA
609 translation may facilitate metabolic adaptations of cancer cells to KIs.

610 Combinations of kinase inhibitors that impinge on **EIF4F** (e.g. MTOR inhibitors) with
611 oncogenic kinase inhibitors (e.g. TKIs) have been explored. The BOLERO-3 clinical trial has
612 tested a combination of everolimus, a rapalog, and trastuzumab, which targets the HER2 receptor
613 (Andre, et al. 2014). Based on the initial results of the BOLOERO-3 trial, it appears that such
614 combination represents a promising therapeutic strategy to target patients with advanced HER2+
615 breast cancer developing resistance to conventional therapy (Andre et al. 2014). However, in a
616 subset of patients, high **EIF4E/EIF4EBP** ratio may result in resistance to
617 trastuzumab/everolimus combinations (Alain et al. 2012a). Notably, alternative possibilities of
618 targeting the translation machinery have been developed (e.g. **EIF4A** inhibitors). These therapies
619 target directly the formation of the **EIF4F** complex and could provide good candidates for
620 combination with kinase inhibitors to manage resistance. They are discussed in more detail in the
621 next section.
622

623 **5. Cancer metabolism and therapeutic implications**

624 Although metabolic reprograming in cancer is thought to provide sufficient therapeutic
625 window to selectively target malignant cells, while not causing excessive toxicity in normal
626 tissues, changes in metabolic and associated translational programs are also linked to the
627 development of drug resistance (Deblois, et al. 2016; Han et al. 2015; Zhao, et al. 2010). For
628 example, sustained MTOR activation observed in SKBr3 lapatinib-resistant cells, leads to
629 dysregulated expression of ESSRA, which mediates lapatinib resistance through increased
630 glutamine metabolism and ROS detoxification (Deblois et al. 2016). Moreover, ESRRA
631 mediates the intrinsic resistance of breast cancer cells to PI3K/MTOR inhibitors (Park, et al.
632 2016). ESRRA regulates the expression of genes that allow utilization of lactate as an energy

source, which enables breast cancer cells to adapt to extended periods of glucose deprivation (Park et al. 2016). Vemurafenib-resistant cells have been shown to reactivate their MAPK signaling pathway and/or to have high MTOR and **EIF4F** activity (Boussemart et al. 2014; Poulikakos, et al. 2011). These cells also uptake glutamine at a faster rate compared to non-resistant cells (Hernandez-Davies, et al. 2015). In addition, vemurafenib resistance induces an oxidative phosphorylation gene program, mitochondrial biogenesis, and increase expression of **PPARGC1A** (Han et al. 2015). Imatinib-resistant chronic myelogenous leukemia (CML) cells have been shown to have increased glycolytic rate and HIF1A-dependent activation of the non-oxidative PPP transketolase enzyme (Zhao et al. 2010). Hence, metabolic reprogramming at least in part mediates the resistance of malignant cells to kinase inhibitors, which is further exacerbated by the seemingly outstandingly plasticity of malignant metabolomes.

Strategies to overcome metabolic adaptations of cancer cells to kinase inhibitors, whereby combinatory drugs are used to disrupt metabolic reprogramming processes which underpin development of resistance, are being developed. For example, the inhibition of ESRRA with compound C29, used in combination with lapatinib, may be effective in treating lapatinib-resistant cells (Deblois et al. 2016). This is because C29 impedes the ESRRA-mediated glutamine addiction that results from lapatinib treatment (Deblois et al. 2016). Metformin is a biguanide drug which is commonly used for treatment of type 2 diabetes (Pollak 2010). Biguanides (Figure 1) induce energetic stress by reducing oxidative phosphorylation through the partial inhibition of complex I of the mitochondrial respiratory chain (Andrzejewski, et al. 2014; Bridges, et al. 2014; Wheaton, et al. 2014). This leads to increased glucose uptake and elevated dependence on glycolysis (Javeshghani, et al. 2012). Hence, there is a rationale for combining BRAF inhibitors (BRAFi), which suppress glycolysis,

656 and biguanides (Zhao et al. 2010). Indeed, phenformin -a more potent inhibitor of mitochondrial
657 complex I- and BRAF inhibitors exhibits synergistic anti-tumorigenic effects in melanoma
658 (Bridges et al. 2014; Yuan, et al. 2013). Furthermore, BRAFi resistant melanoma cells have an
659 increased reliance on glutaminolysis, as they were shown to be more sensitive to glutamine
660 starvation and glutaminase inhibitors compared to BRAFi-sensitive cells (Baenke, et al. 2016;
661 Hernandez-Davies et al. 2015). These examples suggest that using drug combinations that alter
662 metabolic adaptations which underlie resistance to KIs may constitute an effective therapeutic
663 strategy.

664 Consistently, combination of phenformin with various kinase inhibitors (lapatinib,
665 vemurafenib, imatinib) results in synergistic anti-proliferative effects, which are paralleled by
666 **MTORC1** inhibition, disruption of the **EIF4F** complex and the downregulation of the
667 translational control of genes involved in non-essential amino acid synthesis (NEAA) (serine,
668 aspartate, asparagine): phosphoglycerate dehydrogenase (**PHGDH**), phosphoserine
669 aminotransferase 1 (**PSAT1**), **PC** and **ASNS** (Hulea *et al*., Cell Metabolism, in press; bioRxiv
670 160879; doi: https://doi.org/10.1101/160879) (Figure 2). However, cells lacking **EIF4EBP1** and
671 **EIF4EBP2**, in which **MTORC1** inhibition is uncoupled from **EIF4F** disassembly, show
672 dramatically reduced sensitivity to the phenformin/KI combinations enzymes (Hulea *et al*., Cell
673 Metabolism, in press; bioRxiv 160879; doi: https://doi.org/10.1101/160879). This at least in part
674 is a consequence of their inability to suppress NEAA biosynthesis and translation of mRNAs
675 encoding corresponding enzymes (Hulea *et al*., Cell Metabolism, in press; bioRxiv 160879; doi:
676 https://doi.org/10.1101/160879). A similar phenomenon was observed in cells depleted of von
677 Hippel–Lindau (VHL) tumor suppressor, the major regulator of **HIF1A** protein stability
678 (Semenza 2007). VHL-null cells maintain high **HIF1A** protein levels under normoxic conditions

679 (Maxwell, et al. 1999) and are less sensitive to the phenformin/KI combinations, at least in part
680 due to changes in the glutamine metabolic program in these cells (Hulea *et al*., Cell Metabolism,
681 in press; bioRxiv 160879; doi: https://doi.org/10.1101/160879). Collectively, these findings
682 emphasize the plasticity of translational and metabolic programs of cancer cells, which allows
683 them to rapidly adapt to therapeutic insults.

684 One way of circumventing the problems associated with plasticity of cancer cells may be
685 a direct targeting of the **EIF4F** complex. **EIF4A** inhibitors (EIF4Ai), but not MTOR inhibitors,
686 lead to specific translational reprogramming, which results in mitochondrial depolarization and
687 cancer cell death (Gandin et al. 2016) (Figure 2). The cytotoxic effect of EIF4Ai is noteworthy,
688 as the effect of MTOR inhibitors on cancer cells is cytostatic (Gandin et al. 2016). The cytostatic
689 effect of MTOR inhibition can be explained by modification in the translational program that
690 reduce both energy production and energy utilization (Morita et al. 2013), leading to metabolic
691 dormancy. These studies highlight the superiority of EIF4Ai and other drugs directly affecting
692 the **EIF4F** complex formation and, in light of what has been discussed above, warrant additional
693 effort into better understanding their effects on cancer cell metabolism. SBI-756 is a compound
694 which was shown to bind to **EIF4G1** and disrupt the **EIF4F** complex independently of the
695 **MTOR/EIF4EBP** axis (Feng, et al. 2015). Most importantly, SBI-756 eradicated BRAF-
696 inhibitor resistant melanoma cells, as well as **EIF4EBP** null cancer cells, which are resistant to
697 MTOR inhibitors (Feng et al. 2015).

698 In the context of combination therapy with drugs inducing energetic stress, therapies
699 resulting in reduced energy consumption, leading to metabolic dormancy and a cytostatic effect,
700 are not very effective. Therefore, in order to effectively kill cancer cells by inducing energetic
701 stress, the ideal drug combination would be one that on one hand reduces energy production, and

on the other hand affects dysregulated oncogenic signal while maintaining energy consumption (e.g. by carrying specific translational reprogramming without greatly affecting global translation levels).

6. Future perspectives

Although significant efforts have been made to therapeutically target cancer metabolism, progress remains limited. It is becoming apparent that intratumor heterogeneity severely hinders the success of therapeutic efforts aiming to target metabolic vulnerabilities. It is likely impossible to develop effective treatments that eliminate the dozens of aberrant signalling pathways that are present within a single resistant tumor. However, considering that the abnormal regulation of mRNA translation, resulting in metabolic reprogramming, is a "final common pathway" downstream of driver mutations, we can assume that therapies that restrain abnormal translation may have utility independent of the nature of upstream drivers. Of particular interest would be the opportunities for synthetic lethality whereby one drug induces a metabolic stress while the other impedes adaptation of cancer cells to that stress. Further research is thus warranted to grasp the full complexity and plasticity of cancer metabolomes.

Declaration of interest:

There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

IT is supported by Junior 2 FRQ-S award and research in our lab pertinent to this review is funded by grants Canadian Cancer Society Research Institute (CCSRI-703816) to IT and MP, Canadian Institutes for Health Research (MOP-363027) to IT, and Terry Fox Research Institute (TFF-116128) to IT and MP.

Acknowledgments:

The authors thank Shannon McLaughlan for the administrative support and Kristofferson Tandoc for the critical reading of the manuscript.

738 **References:**

739 Accili D & Arden KC 2004 FoxOs at the crossroads of cellular metabolism, differentiation, and
740 transformation. *Cell* **117** 421-426.
741 Agirrezabala X & Frank J 2009 Elongation in translation as a dynamic interaction among the
742 ribosome, tRNA, and elongation factors EF-G and EF-Tu. *Q Rev Biophys* **42** 159-200.
743 Alain T, Morita M, Fonseca BD, Yanagiya A, Siddiqui N, Bhat M, Zammit D, Marcus V,
744 Metrakos P, Voyer LA, et al. 2012a eIF4E/4E-BP ratio predicts the efficacy of mTOR targeted
745 therapies. *Cancer Res* **72** 6468-6476.
746 Alain T, Sonenberg N & Topisirovic I 2012b mTOR inhibitor efficacy is determined by the
747 eIF4E/4E-BP ratio. *Oncotarget* **3** 1491-1492.
748 Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB & Cohen P 1997
749 Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and
750 activates protein kinase Balpha. *Curr Biol* **7** 261-269.
751 Alkalaeva EZ, Pisarev AV, Frolova LY, Kisselev LL & Pestova TV 2006 In vitro reconstitution
752 of eukaryotic translation reveals cooperativity between release factors eRF1 and eRF3. *Cell* **125**
753 1125-1136.
754 Andre F, O'Regan R, Ozguroglu M, Toi M, Xu B, Jerusalem G, Masuda N, Wilks S, Arena F,
755 Isaacs C, et al. 2014 Everolimus for women with trastuzumab-resistant, HER2-positive,
756 advanced breast cancer (BOLERO-3): a randomised, double-blind, placebo-controlled phase 3
757 trial. *Lancet Oncol* **15** 580-591.
758 Andrzejewski S, Gravel SP, Pollak M & St-Pierre J 2014 Metformin directly acts on
759 mitochondria to alter cellular bioenergetics. *Cancer Metab* **2** 12.
760 Audet-Walsh E, Dufour CR, Yee T, Zouanat FZ, Yan M, Kalloghlian G, Vernier M, Caron M,
761 Bourque G, Scarlata E, et al. 2017 Nuclear mTOR acts as a transcriptional integrator of the
762 androgen signaling pathway in prostate cancer. *Genes Dev*.
763 Baenke F, Chaneton B, Smith M, Van Den Broek N, Hogan K, Tang H, Viros A, Martin M,
764 Galbraith L, Girotti MR, et al. 2016 Resistance to BRAF inhibitors induces glutamine
765 dependency in melanoma cells. *Mol Oncol* **10** 73-84.
766 Banerjee P, Ahmad MF, Grove JR, Kozlosky C, Price DJ & Avruch J 1990 Molecular structure
767 of a major insulin/mitogen-activated 70-kDa S6 protein kinase. *Proc Natl Acad Sci U S A* **87**
768 8550-8554.
769 Bar-Peled L, Chantranupong L, Cherniack AD, Chen WW, Ottina KA, Grabiner BC, Spear ED,
770 Carter SL, Meyerson M & Sabatini DM 2013 A Tumor suppressor complex with GAP activity
771 for the Rag GTPases that signal amino acid sufficiency to mTORC1. *Science* **340** 1100-1106.
772 Bar-Peled L, Schweitzer LD, Zoncu R & Sabatini DM 2012 Ragulator is a GEF for the rag
773 GTPases that signal amino acid levels to mTORC1. *Cell* **150** 1196-1208.
774 Ben-Sahra I, Howell JJ, Asara JM & Manning BD 2013 Stimulation of de novo pyrimidine
775 synthesis by growth signaling through mTOR and S6K1. *Science* **339** 1323-1328.
776 Ben-Sahra I, Hoxhaj G, Ricoult SJH, Asara JM & Manning BD 2016 mTORC1 induces purine
777 synthesis through control of the mitochondrial tetrahydrofolate cycle. *Science* **351** 728-733.
778 Benjamin D, Colombi M, Moroni C & Hall MN 2011 Rapamycin passes the torch: a new
779 generation of mTOR inhibitors. *Nat Rev Drug Discov* **10** 868-880.
780 Berwick DC, Hers I, Heesom KJ, Moule SK & Tavare JM 2002 The identification of ATP-
781 citrate lyase as a protein kinase B (Akt) substrate in primary adipocytes. *J Biol Chem* **277** 33895-
782 33900.

783 Betz C, Stracka D, Prescianotto-Baschong C, Frieden M, Demaurex N & Hall MN 2013 Feature
784 Article: mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum
785 membranes (MAM) regulates mitochondrial physiology. *Proc Natl Acad Sci U S A* **110** 12526-
786 12534.
787 Boren J, Cascante M, Marin S, Comin-Anduix B, Centelles JJ, Lim S, Bassilian S, Ahmed S, Lee
788 WN & Boros LG 2001 Gleevec (STI571) influences metabolic enzyme activities and glucose
789 carbon flow toward nucleic acid and fatty acid synthesis in myeloid tumor cells. *J Biol Chem* **276**
790 37747-37753.
791 Boussemart L, Malka-Mahieu H, Girault I, Allard D, Hemmingsson O, Tomasic G, Thomas M,
792 Basmadjian C, Ribeiro N, Thuaud F, et al. 2014 eIF4F is a nexus of resistance to anti-BRAF and
793 anti-MEK cancer therapies. *Nature*.
794 Brady SW, Zhang J, Tsai MH & Yu D 2015 PI3K-independent mTOR activation promotes
795 lapatinib resistance and IAP expression that can be effectively reversed by mTOR and Hsp90
796 inhibition. *Cancer Biol Ther* **16** 402-411.
797 Bridges HR, Jones AJ, Pollak MN & Hirst J 2014 Effects of metformin and other biguanides on
798 oxidative phosphorylation in mitochondria. *Biochem J* **462** 475-487.
799 Brown NF, Stefanovic-Racic M, Sipula IJ & Perdomo G 2007 The mammalian target of
800 rapamycin regulates lipid metabolism in primary cultures of rat hepatocytes. *Metabolism* **56**
801 1500-1507.
802 Browne GJ, Finn SG & Proud CG 2004 Stimulation of the AMP-activated protein kinase leads to
803 activation of eukaryotic elongation factor 2 kinase and to its phosphorylation at a novel site,
804 serine 398. *J Biol Chem* **279** 12220-12231.
805 Browne GJ & Proud CG 2004 A novel mTOR-regulated phosphorylation site in elongation
806 factor 2 kinase modulates the activity of the kinase and its binding to calmodulin. *Mol Cell Biol*
807 **24** 2986-2997.
808 Brugarolas J, Lei K, Hurley RL, Manning BD, Reiling JH, Hafen E, Witters LA, Ellisen LW &
809 Kaelin WG, Jr. 2004 Regulation of mTOR function in response to hypoxia by REDD1 and the
810 TSC1/TSC2 tumor suppressor complex. *Genes Dev* **18** 2893-2904.
811 Brunn GJ, Hudson CC, Sekulic A, Williams JM, Hosoi H, Houghton PJ, Lawrence JC, Jr. &
812 Abraham RT 1997 Phosphorylation of the translational repressor PHAS-I by the mammalian
813 target of rapamycin. *Science* **277** 99-101.
814 Buttgereit F & Brand MD 1995 A hierarchy of ATP-consuming processes in mammalian cells.
815 *Biochem J* **312 (Pt 1)** 163-167.
816 Byrnes KW, DeBenedetti A, Holm NT, Luke J, Nunez J, Chu QD, Meschonat C, Abreo F,
817 Johnson LW & Li BD 2007 Correlation of TLK1B in elevation and recurrence in doxorubicin-
818 treated breast cancer patients with high eIF4E overexpression. *J Am Coll Surg* **204** 925-933;
819 discussion 933-924.
820 Cantley LC 2002 The phosphoinositide 3-kinase pathway. *Science* **296** 1655-1657.
821 Carlberg U, Nilsson A & Nygard O 1990 Functional properties of phosphorylated elongation
822 factor 2. *Eur J Biochem* **191** 639-645.
823 Chang CH, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, Chen Q, Gindin M, Gubin
824 MM, van der Windt GJ, et al. 2015 Metabolic Competition in the Tumor Microenvironment Is a
825 Driver of Cancer Progression. *Cell* **162** 1229-1241.
826 Chantranupong L, Scaria SM, Saxton RA, Gygi MP, Shen K, Wyant GA, Wang T, Harper JW,
827 Gygi SP & Sabatini DM 2016 The CASTOR Proteins Are Arginine Sensors for the mTORC1
828 Pathway. *Cell* **165** 153-164.

829 Chauvin C, Koka V, Nouschi A, Mieulet V, Hoareau-Aveilla C, Dreazen A, Cagnard N,
830 Carpentier W, Kiss T, Meyuhas O, et al. 2014 Ribosomal protein S6 kinase activity controls the
831 ribosome biogenesis transcriptional program. *Oncogene* **33** 474-483.
832 Cheng JQ, Lindsley CW, Cheng GZ, Yang H & Nicosia SV 2005 The Akt/PKB pathway:
833 molecular target for cancer drug discovery. *Oncogene* **24** 7482-7492.
834 Cho HJ, Park J, Lee HW, Lee YS & Kim JB 2004 Regulation of adipocyte differentiation and
835 insulin action with rapamycin. *Biochem Biophys Res Commun* **321** 942-948.
836 Choi SY, Collins CC, Gout PW & Wang Y 2013 Cancer-generated lactic acid: a regulatory,
837 immunosuppressive metabolite? *J Pathol* **230** 350-355.
838 Cross DA, Alessi DR, Cohen P, Andjelkovich M & Hemmings BA 1995 Inhibition of glycogen
839 synthase kinase-3 by insulin mediated by protein kinase B. *Nature* **378** 785-789.
840 Csibi A, Fendt SM, Li C, Poulogiannis G, Choo AY, Chapski DJ, Jeong SM, Dempsey JM,
841 Parkhitko A, Morrison T, et al. 2013 The mTORC1 pathway stimulates glutamine metabolism
842 and cell proliferation by repressing SIRT4. *Cell* **153** 840-854.
843 Csibi A, Lee G, Yoon SO, Tong H, Ilter D, Elia I, Fendt SM, Roberts TM & Blenis J 2014 The
844 mTORC1/S6K1 pathway regulates glutamine metabolism through the eIF4B-dependent control
845 of c-Myc translation. *Curr Biol* **24** 2274-2280.
846 Cunningham JT, Moreno MV, Lodi A, Ronen SM & Ruggero D 2014 Protein and nucleotide
847 biosynthesis are coupled by a single rate-limiting enzyme, PRPS2, to drive cancer. *Cell* **157**
848 1088-1103.
849 Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK & Puigserver P 2007 mTOR
850 controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex.
851 *Nature* **450** 736-740.
852 Cybulski N, Polak P, Auwerx J, Ruegg MA & Hall MN 2009 mTOR complex 2 in adipose tissue
853 negatively controls whole-body growth. *Proc Natl Acad Sci U S A* **106** 9902-9907.
854 Dang CV, Hamaker M, Sun P, Le A & Gao P 2011 Therapeutic targeting of cancer cell
855 metabolism. *J Mol Med (Berl)* **89** 205-212.
856 De Benedetti A & Graff JR 2004 eIF-4E expression and its role in malignancies and metastases.
857 *Oncogene* **23** 3189-3199.
858 DeBerardinis RJ, Lum JJ, Hatzivassiliou G & Thompson CB 2008 The biology of cancer:
859 metabolic reprogramming fuels cell growth and proliferation. *Cell Metab* **7** 11-20.
860 DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S & Thompson CB 2007
861 Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds
862 the requirement for protein and nucleotide synthesis. *Proc Natl Acad Sci U S A* **104** 19345-
863 19350.
864 Deblois G, Smith HW, Tam IS, Gravel SP, Caron M, Savage P, Labbe DP, Begin LR, Tremblay
865 ML, Park M, et al. 2016 ERRalpha mediates metabolic adaptations driving lapatinib resistance in
866 breast cancer. *Nat Commun* **7** 12156.
867 Delgado-Goni T, Miniotis MF, Wantuch S, Parkes HG, Marais R, Workman P, Leach MO &
868 Beloueche-Babari M 2016 The BRAF Inhibitor Vemurafenib Activates Mitochondrial
869 Metabolism and Inhibits Hyperpolarized Pyruvate-Lactate Exchange in BRAF-Mutant Human
870 Melanoma Cells. *Mol Cancer Ther* **15** 2987-2999.
871 Dennis MD, Jefferson LS & Kimball SR 2012 Role of p70S6K1-mediated phosphorylation of
872 eIF4B and PDCD4 proteins in the regulation of protein synthesis. *J Biol Chem* **287** 42890-42899.

873 Deprez J, Vertommen D, Alessi DR, Hue L & Rider MH 1997 Phosphorylation and activation of
874 heart 6-phosphofructo-2-kinase by protein kinase B and other protein kinases of the insulin
875 signaling cascades. *J Biol Chem* **272** 17269-17275.
876 Dever TE & Green R 2012 The elongation, termination, and recycling phases of translation in
877 eukaryotes. *Cold Spring Harb Perspect Biol* **4** a013706.
878 DeYoung MP, Horak P, Sofer A, Sgroi D & Ellisen LW 2008 Hypoxia regulates TSC1/2-mTOR
879 signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling. *Genes Dev* **22** 239-
880 251.
881 Dibble CC, Elis W, Menon S, Qin W, Klekota J, Asara JM, Finan PM, Kwiatkowski DJ, Murphy
882 LO & Manning BD 2012 TBC1D7 is a third subunit of the TSC1-TSC2 complex upstream of
883 mTORC1. *Mol Cell* **47** 535-546.
884 Dickens F & Williamson DH 1956 Pentose phosphate isomerase and epimerase from animal
885 tissues. *Biochem J* **64** 567-578.
886 Dodd KM, Yang J, Shen MH, Sampson JR & Tee AR 2015 mTORC1 drives HIF-1alpha and
887 VEGF-A signalling via multiple mechanisms involving 4E-BP1, S6K1 and STAT3. *Oncogene*
888 **34** 2239-2250.
889 Domchek SM, Auger KR, Chatterjee S, Burke TR, Jr. & Shoelson SE 1992 Inhibition of SH2
890 domain/phosphoprotein association by a nonhydrolyzable phosphonopeptide. *Biochemistry* **31**
891 9865-9870.
892 Dorrello NV, Peschiaroli A, Guardavaccaro D, Colburn NH, Sherman NE & Pagano M 2006
893 S6K1- and betaTRCP-mediated degradation of PDCD4 promotes protein translation and cell
894 growth. *Science* **314** 467-471.
895 Dowling RJ, Topisirovic I, Alain T, Bidinosti M, Fonseca BD, Petroulakis E, Wang X, Larsson
896 O, Selvaraj A, Liu Y, et al. 2010 mTORC1-mediated cell proliferation, but not cell growth,
897 controlled by the 4E-BPs. *Science* **328** 1172-1176.
898 Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, Triantafellow E, Ma Q,
899 Gorski R, Cleaver S, et al. 2010 Activation of a metabolic gene regulatory network downstream
900 of mTOR complex 1. *Mol Cell* **39** 171-183.
901 Efeyan A, Zoncu R, Chang S, Gumper I, Snitkin H, Wolfson RL, Kirak O, Sabatini DD &
902 Sabatini DM 2013 Regulation of mTORC1 by the Rag GTPases is necessary for neonatal
903 autophagy and survival. *Nature* **493** 679-683.
904 Elfakess R, Sinvani H, Haimov O, Svitkin Y, Sonenberg N & Dikstein R 2011 Unique
905 translation initiation of mRNAs-containing TISU element. *Nucleic Acids Res* **39** 7598-7609.
906 Elstrom RL, Bauer DE, Buzzai M, Karnauskas R, Harris MH, Plas DR, Zhuang H, Cinalli RM,
907 Alavi A, Rudin CM, et al. 2004 Akt stimulates aerobic glycolysis in cancer cells. *Cancer Res* **64**
908 3892-3899.
909 Faes S, Demartines N & Dormond O 2017 Resistance to mTORC1 Inhibitors in Cancer Therapy:
910 From Kinase Mutations to Intratumoral Heterogeneity of Kinase Activity. *Oxid Med Cell Longev*
911 **2017** 1726078.
912 Fasolo A & Sessa C 2008 mTOR inhibitors in the treatment of cancer. *Expert Opin Investig*
913 *Drugs* **17** 1717-1734.
914 Faubert B, Li KY, Cai L, Hensley CT, Kim J, Zacharias LG, Yang C, Do QN, Doucette S,
915 Burguete D, et al. 2017 Lactate Metabolism in Human Lung Tumors. *Cell* **171** 358-371 e359.
916 Feng Y, Pinkerton AB, Hulea L, Zhang T, Davies MA, Grotegut S, Cheli Y, Yin H, Lau E, Kim
917 H, et al. 2015 SBI-0640756 Attenuates the Growth of Clinically Unresponsive Melanomas by
918 Disrupting the eIF4F Translation Initiation Complex. *Cancer Res* **75** 5211-5218.

Flynn A, Vries RG & Proud CG 1997 Signalling pathways which regulate eIF4E. *Biochem Soc Trans* **25** 192S.

Fonseca BD, Zakaria C, Jia JJ, Graber TE, Svitkin Y, Tahmasebi S, Healy D, Hoang HD, Jensen JM, Diao IT, et al. 2015 La-related Protein 1 (LARP1) Represses Terminal Oligopyrimidine (TOP) mRNA Translation Downstream of mTOR Complex 1 (mTORC1). *J Biol Chem* **290** 15996-16020.

Fraenkel M, Ketzinel-Gilad M, Ariav Y, Pappo O, Karaca M, Castel J, Berthault MF, Magnan C, Cerasi E, Kaiser N, et al. 2008 mTOR inhibition by rapamycin prevents beta-cell adaptation to hyperglycemia and exacerbates the metabolic state in type 2 diabetes. *Diabetes* **57** 945-957.

Fresno Vara JA, Casado E, de Castro J, Cejas P, Belda-Iniesta C & Gonzalez-Baron M 2004 PI3K/Akt signalling pathway and cancer. *Cancer Treat Rev* **30** 193-204.

Furic L, Rong L, Larsson O, Koumakpayi IH, Yoshida K, Brueschke A, Petroulakis E, Robichaud N, Pollak M, Gaboury LA, et al. 2010 eIF4E phosphorylation promotes tumorigenesis and is associated with prostate cancer progression. *Proc Natl Acad Sci U S A* **107** 14134-14139.

G. P 1896 Beitrag zur histologie und aetiologie der carcinoma. histologische und experimentelle untersuchungen. . *Beitr. Pathol. Anat. Allgem. Pathol.* **142** 1–193.

Gandin V, Masvidal L, Hulea L, Gravel SP, Cargnello M, McLaughlan S, Cai Y, Balanathan P, Morita M, Rajakumar A, et al. 2016 nanoCAGE reveals 5' UTR features that define specific modes of translation of functionally related MTOR-sensitive mRNAs. *Genome Res* **26** 636-648.

Gentilella A, Kozma SC & Thomas G 2015 A liaison between mTOR signaling, ribosome biogenesis and cancer. *Biochim Biophys Acta* **1849** 812-820.

Geyer CE, Forster J, Lindquist D, Chan S, Romieu CG, Pienkowski T, Jagiello-Gruszfeld A, Crown J, Chan A, Kaufman B, et al. 2006 Lapatinib plus capecitabine for HER2-positive advanced breast cancer. *N Engl J Med* **355** 2733-2743.

Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R & Sonenberg N 1999 Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev* **13** 1422-1437.

Gingras AC, Raught B, Gygi SP, Niedzwiecka A, Miron M, Burley SK, Polakiewicz RD, Wyslouch-Cieszynska A, Aebersold R & Sonenberg N 2001 Hierarchical phosphorylation of the translation inhibitor 4E-BP1. *Genes Dev* **15** 2852-2864.

Glaser L & Brown DH 1955 Purification and properties of d-glucose-6-phosphate dehydrogenase. *J Biol Chem* **216** 67-79.

Gordan JD, Thompson CB & Simon MC 2007 HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. *Cancer Cell* **12** 108-113.

Gottschalk S, Anderson N, Hainz C, Eckhardt SG & Serkova NJ 2004 Imatinib (STI571)-mediated changes in glucose metabolism in human leukemia BCR-ABL-positive cells. *Clin Cancer Res* **10** 6661-6668.

Guan BJ, van Hoef V, Jobava R, Elroy-Stein O, Valasek LS, Cargnello M, Gao XH, Krokowski D, Merrick WC, Kimball SR, et al. 2017 A Unique ISR Program Determines Cellular Responses to Chronic Stress. *Mol Cell* **68** 885-900 e886.

Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, Turk BE & Shaw RJ 2008 AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* **30** 214-226.

963 Hagiwara A, Cornu M, Cybulski N, Polak P, Betz C, Trapani F, Terracciano L, Heim MH,
964 Ruegg MA & Hall MN 2012 Hepatic mTORC2 activates glycolysis and lipogenesis through Akt,
965 glucokinase, and SREBP1c. *Cell Metab* **15** 725-738.
966 Hagner PR, Schneider A & Gartenhaus RB 2010 Targeting the translational machinery as a
967 novel treatment strategy for hematologic malignancies. *Blood* **115** 2127-2135.
968 Han J, Li E, Chen L, Zhang Y, Wei F, Liu J, Deng H & Wang Y 2015 The CREB coactivator
969 CRTC2 controls hepatic lipid metabolism by regulating SREBP1. *Nature* **524** 243-246.
970 Hanahan D & Weinberg RA 2011 Hallmarks of cancer: the next generation. *Cell* **144** 646-674.
971 Harding MW, Galat A, Uehling DE & Schreiber SL 1989 A receptor for the immunosuppressant
972 FK506 is a cis-trans peptidyl-prolyl isomerase. *Nature* **341** 758-760.
973 Hay N & Sonenberg N 2004 Upstream and downstream of mTOR. *Genes Dev* **18** 1926-1945.
974 Hernandez-Davies JE, Tran TQ, Reid MA, Rosales KR, Lowman XH, Pan M, Moriceau G,
975 Yang Y, Wu J, Lo RS, et al. 2015 Vemurafenib resistance reprograms melanoma cells towards
976 glutamine dependence. *J Transl Med* **13** 210.
977 Hershey JW, Sonenberg N & Mathews MB 2012 Principles of translational control: an overview.
978 *Cold Spring Harb Perspect Biol* **4**.
979 Hinnebusch AG 2014 The scanning mechanism of eukaryotic translation initiation. *Annu Rev*
980 *Biochem* **83** 779-812.
981 Hirao T, Yamaguchi M, Kikuya M, Chibana H, Ito K & Aoki S 2018 Altered intracellular
982 signaling by imatinib increases the anti-cancer effects of tyrosine kinase inhibitors in chronic
983 myelogenous leukemia cells. *Cancer Sci* **109** 121-131.
984 Ho PC, Bihuniak JD, Macintyre AN, Staron M, Liu X, Amezquita R, Tsui YC, Cui G, Micevic
985 G, Perales JC, et al. 2015 Phosphoenolpyruvate Is a Metabolic Checkpoint of Anti-tumor T Cell
986 Responses. *Cell* **162** 1217-1228.
987 Holz MK, Ballif BA, Gygi SP & Blenis J 2005 mTOR and S6K1 mediate assembly of the
988 translation preinitiation complex through dynamic protein interchange and ordered
989 phosphorylation events. *Cell* **123** 569-580.
990 Hong S, Freeberg MA, Han T, Kamath A, Yao Y, Fukuda T, Suzuki T, Kim JK & Inoki K 2017
991 LARP1 functions as a molecular switch for mTORC1-mediated translation of an essential class
992 of mRNAs. *Elife* **6**.
993 Horecker BL, Smyrniotis PZ & Seegmiller JE 1951 The enzymatic conversion of 6-
994 phosphogluconate to ribulose-5-phosphate and ribose-5-phosphate. *J Biol Chem* **193** 383-396.
995 Horton JD, Goldstein JL & Brown MS 2002 SREBPs: transcriptional mediators of lipid
996 homeostasis. *Cold Spring Harb Symp Quant Biol* **67** 491-498.
997 Hove-Jensen B 1989 Phosphoribosylpyrophosphate (PRPP)-less mutants of Escherichia coli.
998 *Mol Microbiol* **3** 1487-1492.
999 Hsieh AC, Liu Y, Edlind MP, Ingolia NT, Janes MR, Sher A, Shi EY, Stumpf CR, Christensen
1000 C, Bonham MJ, et al. 2012 The translational landscape of mTOR signalling steers cancer
1001 initiation and metastasis. *Nature* **485** 55-61.
1002 Hsu PP & Sabatini DM 2008 Cancer cell metabolism: Warburg and beyond. *Cell* **134** 703-707.
1003 Ilic N, Utermark T, Widlund HR & Roberts TM 2011 PI3K-targeted therapy can be evaded by
1004 gene amplification along the MYC-eukaryotic translation initiation factor 4E (eIF4E) axis. *Proc*
1005 *Natl Acad Sci U S A* **108** E699-708.
1006 Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, Yang Q, Bennett C, Harada Y,
1007 Stankunas K, et al. 2006 TSC2 integrates Wnt and energy signals via a coordinated
1008 phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell* **126** 955-968.

1009 Inoki K, Zhu T & Guan KL 2003 TSC2 mediates cellular energy response to control cell growth
1010 and survival. *Cell* **115** 577-590.
1011 Jan E, Mohr I & Walsh D 2016 A Cap-to-Tail Guide to mRNA Translation Strategies in Virus-
1012 Infected Cells. *Annu Rev Virol* **3** 283-307.
1013 Javeshghani S, Zakikhani M, Austin S, Bazile M, Blouin MJ, Topisirovic I, St-Pierre J & Pollak
1014 MN 2012 Carbon source and myc expression influence the antiproliferative actions of
1015 metformin. *Cancer Res* **72** 6257-6267.
1016 Jefferies HB, Fumagalli S, Dennis PB, Reinhard C, Pearson RB & Thomas G 1997 Rapamycin
1017 suppresses 5'TOP mRNA translation through inhibition of p70s6k. *EMBO J* **16** 3693-3704.
1018 Jefferies HB, Reinhard C, Kozma SC & Thomas G 1994 Rapamycin selectively represses
1019 translation of the "polypyrimidine tract" mRNA family. *Proc Natl Acad Sci U S A* **91** 4441-4445.
1020 Jessani N, Humphrey M, McDonald WH, Niessen S, Masuda K, Gangadharan B, Yates JR, 3rd,
1021 Mueller BM & Cravatt BF 2004 Carcinoma and stromal enzyme activity profiles associated with
1022 breast tumor growth in vivo. *Proc Natl Acad Sci U S A* **101** 13756-13761.
1023 Johnson LF, Levis R, Abelson HT, Green H & Penman S 1976 Changes in RNA in relation to
1024 growth of the fibroblast. IV. Alterations in theproduction and processing of mRNA and rRNA in
1025 resting and growing cells. *J Cell Biol* **71** 933-938.
1026 Jung J, Genau HM & Behrends C 2015 Amino Acid-Dependent mTORC1 Regulation by the
1027 Lysosomal Membrane Protein SLC38A9. *Mol Cell Biol* **35** 2479-2494.
1028 Kahn BB, Alquier T, Carling D & Hardie DG 2005 AMP-activated protein kinase: ancient
1029 energy gauge provides clues to modern understanding of metabolism. *Cell Metab* **1** 15-25.
1030 Kauffmann-Zeh A, Rodriguez-Viciana P, Ulrich E, Gilbert C, Coffer P, Downward J & Evan G
1031 1997 Suppression of c-Myc-induced apoptosis by Ras signalling through PI(3)K and PKB.
1032 *Nature* **385** 544-548.
1033 Keith B, Johnson RS & Simon MC 2011 HIF1alpha and HIF2alpha: sibling rivalry in hypoxic
1034 tumour growth and progression. *Nat Rev Cancer* **12** 9-22.
1035 Kevil CG, De Benedetti A, Payne DK, Coe LL, Laroux FS & Alexander JS 1996 Translational
1036 regulation of vascular permeability factor by eukaryotic initiation factor 4E: implications for
1037 tumor angiogenesis. *Int J Cancer* **65** 785-790.
1038 Kiel MC, Kaji H & Kaji A 2007 Ribosome recycling: An essential process of protein synthesis.
1039 *Biochem Mol Biol Educ* **35** 40-44.
1040 Kim E, Goraksha-Hicks P, Li L, Neufeld TP & Guan KL 2008 Regulation of TORC1 by Rag
1041 GTPases in nutrient response. *Nat Cell Biol* **10** 935-945.
1042 Kim J, Kundu M, Viollet B & Guan KL 2011 AMPK and mTOR regulate autophagy through
1043 direct phosphorylation of Ulk1. *Nat Cell Biol* **13** 132-141.
1044 Kim JE & Chen J 2004 regulation of peroxisome proliferator-activated receptor-gamma activity
1045 by mammalian target of rapamycin and amino acids in adipogenesis. *Diabetes* **53** 2748-2756.
1046 Kim JW, Gao P, Liu YC, Semenza GL & Dang CV 2007 Hypoxia-inducible factor 1 and
1047 dysregulated c-Myc cooperatively induce vascular endothelial growth factor and metabolic
1048 switches hexokinase 2 and pyruvate dehydrogenase kinase 1. *Mol Cell Biol* **27** 7381-7393.
1049 Knauf U, Tschopp C & Gram H 2001 Negative regulation of protein translation by mitogen-
1050 activated protein kinase-interacting kinases 1 and 2. *Mol Cell Biol* **21** 5500-5511.
1051 Koh YK, Lee MY, Kim JW, Kim M, Moon JS, Lee YJ, Ahn YH & Kim KS 2008 Lipin1 is a key
1052 factor for the maturation and maintenance of adipocytes in the regulatory network with
1053 CCAAT/enhancer-binding protein alpha and peroxisome proliferator-activated receptor gamma
1054 2. *J Biol Chem* **283** 34896-34906.

1055 Kohn AD, Summers SA, Birnbaum MJ & Roth RA 1996 Expression of a constitutively active
1056 Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4
1057 translocation. *J Biol Chem* **271** 31372-31378.
1058 Koromilas AE, Lazaris-Karatzas A & Sonenberg N 1992 mRNAs containing extensive
1059 secondary structure in their 5' non-coding region translate efficiently in cells overexpressing
1060 initiation factor eIF-4E. *EMBO J* **11** 4153-4158.
1061 Krall AS, Xu S, Graeber TG, Braas D & Christofk HR 2016 Asparagine promotes cancer cell
1062 proliferation through use as an amino acid exchange factor. *Nat Commun* **7** 11457.
1063 Lamming DW, Ye L, Katajisto P, Goncalves MD, Saitoh M, Stevens DM, Davis JG, Salmon
1064 AB, Richardson A, Ahima RS, et al. 2012 Rapamycin-induced insulin resistance is mediated by
1065 mTORC2 loss and uncoupled from longevity. *Science* **335** 1638-1643.
1066 Laplante M & Sabatini DM 2009a An emerging role of mTOR in lipid biosynthesis. *Curr Biol*
1067 **19** R1046-1052.
1068 Laplante M & Sabatini DM 2009b mTOR signaling at a glance. *J Cell Sci* **122** 3589-3594.
1069 Laplante M & Sabatini DM 2012 mTOR signaling in growth control and disease. *Cell* **149** 274-
1070 293.
1071 Larsson O, Li S, Issaenko OA, Avdulov S, Peterson M, Smith K, Bitterman PB & Polunovsky
1072 VA 2007 Eukaryotic translation initiation factor 4E induced progression of primary human
1073 mammary epithelial cells along the cancer pathway is associated with targeted translational
1074 deregulation of oncogenic drivers and inhibitors. *Cancer Res* **67** 6814-6824.
1075 Larsson O, Morita M, Topisirovic I, Alain T, Blouin MJ, Pollak M & Sonenberg N 2012 Distinct
1076 perturbation of the translatome by the antidiabetic drug metformin. *Proc Natl Acad Sci U S A*
1077 **109** 8977-8982.
1078 Larsson O, Perlman DM, Fan D, Reilly CS, Peterson M, Dahlgren C, Liang Z, Li S, Polunovsky
1079 VA, Wahlestedt C, et al. 2006 Apoptosis resistance downstream of eIF4E: posttranscriptional
1080 activation of an anti-apoptotic transcript carrying a consensus hairpin structure. *Nucleic Acids*
1081 *Res* **34** 4375-4386.
1082 Le Bacquer O, Petroulakis E, Paglialunga S, Poulin F, Richard D, Cianflone K & Sonenberg N
1083 2007 Elevated sensitivity to diet-induced obesity and insulin resistance in mice lacking 4E-BP1
1084 and 4E-BP2. *J Clin Invest* **117** 387-396.
1085 Lemmon MA & Schlessinger J 2010 Cell signaling by receptor tyrosine kinases. *Cell* **141** 1117-
1086 1134.
1087 Li J, Kim SG & Blenis J 2014a Rapamycin: one drug, many effects. *Cell Metab* **19** 373-379.
1088 Li JJ, Bickel PJ & Biggin MD 2014b System wide analyses have underestimated protein
1089 abundances and the importance of transcription in mammals. *PeerJ* **2** e270.
1090 Li S, Oh YT, Yue P, Khuri FR & Sun SY 2016 Inhibition of mTOR complex 2 induces
1091 GSK3/FBXW7-dependent degradation of sterol regulatory element-binding protein 1 (SREBP1)
1092 and suppresses lipogenesis in cancer cells. *Oncogene* **35** 642-650.
1093 Liu P, Cheng H, Roberts TM & Zhao JJ 2009 Targeting the phosphoinositide 3-kinase pathway
1094 in cancer. *Nat Rev Drug Discov* **8** 627-644.
1095 Locasale JW & Cantley LC 2011 Metabolic flux and the regulation of mammalian cell growth.
1096 *Cell Metab* **14** 443-451.
1097 Long X, Lin Y, Ortiz-Vega S, Yonezawa K & Avruch J 2005 Rheb binds and regulates the
1098 mTOR kinase. *Curr Biol* **15** 702-713.
1099 Lunt SY & Vander Heiden MG 2011 Aerobic glycolysis: meeting the metabolic requirements of
1100 cell proliferation. *Annu Rev Cell Dev Biol* **27** 441-464.

1101 Malanga D, Scrima M, De Marco C, Fabiani F, De Rosa N, De Gisi S, Malara N, Savino R,
1102 Rocco G, Chiappetta G, et al. 2008 Activating E17K mutation in the gene encoding the protein
1103 kinase AKT1 in a subset of squamous cell carcinoma of the lung. *Cell Cycle* **7** 665-669.
1104 Mamane Y, Petroulakis E, Rong L, Yoshida K, Ler LW & Sonenberg N 2004 eIF4E--from
1105 translation to transformation. *Oncogene* **23** 3172-3179.
1106 Martelli AM, Chiarini F, Evangelisti C, Cappellini A, Buontempo F, Bressanin D, Fini M &
1107 McCubrey JA 2012 Two hits are better than one: targeting both phosphatidylinositol 3-kinase
1108 and mammalian target of rapamycin as a therapeutic strategy for acute leukemia treatment.
1109 *Oncotarget* **3** 371-394.
1110 Martelli AM, Evangelisti C, Chappell W, Abrams SL, Basecke J, Stivala F, Donia M, Fagone P,
1111 Nicoletti F, Libra M, et al. 2011 Targeting the translational apparatus to improve leukemia
1112 therapy: roles of the PI3K/PTEN/Akt/mTOR pathway. *Leukemia* **25** 1064-1079.
1113 Masui K, Cavenee WK & Mischel PS 2014 mTORC2 in the center of cancer metabolic
1114 reprogramming. *Trends Endocrinol Metab* **25** 364-373.
1115 Masui K, Tanaka K, Akhavan D, Babic I, Gini B, Matsutani T, Iwanami A, Liu F, Villa GR, Gu
1116 Y, et al. 2013 mTOR complex 2 controls glycolytic metabolism in glioblastoma through FoxO
1117 acetylation and upregulation of c-Myc. *Cell Metab* **18** 726-739.
1118 Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, Wykoff CC,
1119 Pugh CW, Maher ER & Ratcliffe PJ 1999 The tumour suppressor protein VHL targets hypoxia-
1120 inducible factors for oxygen-dependent proteolysis. *Nature* **399** 271-275.
1121 Memmott RM & Dennis PA 2009 Akt-dependent and -independent mechanisms of mTOR
1122 regulation in cancer. *Cell Signal* **21** 656-664.
1123 Menendez JA & Lupu R 2007 Fatty acid synthase and the lipogenic phenotype in cancer
1124 pathogenesis. *Nat Rev Cancer* **7** 763-777.
1125 Meyuhas O & Dreazen A 2009 Ribosomal protein S6 kinase from TOP mRNAs to cell size.
1126 *Prog Mol Biol Transl Sci* **90** 109-153.
1127 Miloslavski R, Cohen E, Avraham A, Iluz Y, Hayouka Z, Kasir J, Mudhasani R, Jones SN,
1128 Cybulski N, Ruegg MA, et al. 2014 Oxygen sufficiency controls TOP mRNA translation via the
1129 TSC-Rheb-mTOR pathway in a 4E-BP-independent manner. *J Mol Cell Biol* **6** 255-266.
1130 Mohr I & Sonenberg N 2012 Host translation at the nexus of infection and immunity. *Cell Host*
1131 *Microbe* **12** 470-483.
1132 Morita M, Gravel SP, Chenard V, Sikstrom K, Zheng L, Alain T, Gandin V, Avizonis D,
1133 Arguello M, Zakaria C, et al. 2013 mTORC1 controls mitochondrial activity and biogenesis
1134 through 4E-BP-dependent translational regulation. *Cell Metab* **18** 698-711.
1135 Morita M, Prudent J, Basu K, Goyon V, Katsumura S, Hulea L, Pearl D, Siddiqui N, Strack S,
1136 McGuirk S, et al. 2017 mTOR Controls Mitochondrial Dynamics and Cell Survival via MTFP1.
1137 *Mol Cell* **67** 922-935 e925.
1138 Myers AP & Cantley LC 2010 Targeting a common collaborator in cancer development. *Sci*
1139 *Transl Med* **2** 48ps45.
1140 Nikiforov MA, Chandriani S, O'Connell B, Petrenko O, Kotenko I, Beavis A, Sedivy JM & Cole
1141 MD 2002 A functional screen for Myc-responsive genes reveals serine
1142 hydroxymethyltransferase, a major source of the one-carbon unit for cell metabolism. *Mol Cell*
1143 *Biol* **22** 5793-5800.
1144 Oh WJ & Jacinto E 2011 mTOR complex 2 signaling and functions. *Cell Cycle* **10** 2305-2316.

1145 Osthus RC, Shim H, Kim S, Li Q, Reddy R, Mukherjee M, Xu Y, Wonsey D, Lee LA & Dang
1146 CV 2000 Deregulation of glucose transporter 1 and glycolytic gene expression by c-Myc. *J Biol*
1147 *Chem* **275** 21797-21800.
1148 Oudejans RCHM, Schoonen WGEJ & Voogt PA 1983 Production of NADPH and acetyl CoA
1149 for lipid biosynthesis in the pyloric caeca of the sea star, Asterias rubens. *Comparative*
1150 *Biochemistry and Physiology Part B: Comparative Biochemistry* **74** 351-355.
1151 Paech F, Bouitbir J & Krahenbuhl S 2017 Hepatocellular Toxicity Associated with Tyrosine
1152 Kinase Inhibitors: Mitochondrial Damage and Inhibition of Glycolysis. *Front Pharmacol* **8** 367.
1153 Park S, Chang CY, Safi R, Liu X, Baldi R, Jasper JS, Anderson GR, Liu T, Rathmell JC,
1154 Dewhirst MW, et al. 2016 ERRalpha-Regulated Lactate Metabolism Contributes to Resistance to
1155 Targeted Therapies in Breast Cancer. *Cell Rep* **15** 323-335.
1156 Patel MR, Jay-Dixon J, Sadiq AA, Jacobson BA & Kratzke RA 2013 Resistance to EGFR-TKI
1157 can be mediated through multiple signaling pathways converging upon cap-dependent translation
1158 in EGFR-wild type NSCLC. *J Thorac Oncol* **8** 1142-1147.
1159 Pause A, Belsham GJ, Gingras AC, Donze O, Lin TA, Lawrence JC, Jr. & Sonenberg N 1994
1160 Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap
1161 function. *Nature* **371** 762-767.
1162 Pende M, Um SH, Mieulet V, Sticker M, Goss VL, Mestan J, Mueller M, Fumagalli S, Kozma
1163 SC & Thomas G 2004 S6K1(-/-)/S6K2(-/-) mice exhibit perinatal lethality and rapamycin-
1164 sensitive 5'-terminal oligopyrimidine mRNA translation and reveal a mitogen-activated protein
1165 kinase-dependent S6 kinase pathway. *Mol Cell Biol* **24** 3112-3124.
1166 Peng T, Golub TR & Sabatini DM 2002 The immunosuppressant rapamycin mimics a starvation-
1167 like signal distinct from amino acid and glucose deprivation. *Mol Cell Biol* **22** 5575-5584.
1168 Peterson TR, Sengupta SS, Harris TE, Carmack AE, Kang SA, Balderas E, Guertin DA, Madden
1169 KL, Carpenter AE, Finck BN, et al. 2011 mTOR complex 1 regulates lipin 1 localization to
1170 control the SREBP pathway. *Cell* **146** 408-420.
1171 Pfeiffer T, Schuster S & Bonhoeffer S 2001 Cooperation and competition in the evolution of
1172 ATP-producing pathways. *Science* **292** 504-507.
1173 Philippe L, Vasseur JJ, Debart F & Thoreen CC 2018 La-related protein 1 (LARP1) repression
1174 of TOP mRNA translation is mediated through its cap-binding domain and controlled by an
1175 adjacent regulatory region. *Nucleic Acids Res* **46** 1457-1469.
1176 Pollak M 2010 Metformin and other biguanides in oncology: advancing the research agenda.
1177 *Cancer Prev Res (Phila)* **3** 1060-1065.
1178 Populo H, Lopes JM & Soares P 2012 The mTOR signalling pathway in human cancer. *Int J Mol*
1179 *Sci* **13** 1886-1918.
1180 Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, Griffiths JR, Chung YL &
1181 Schulze A 2008 SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell
1182 growth. *Cell Metab* **8** 224-236.
1183 Porta C, Paglino C & Mosca A 2014 Targeting PI3K/Akt/mTOR Signaling in Cancer. *Front*
1184 *Oncol* **4** 64.
1185 Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, Shi H, Atefi M, Titz B,
1186 Gabay MT, et al. 2011 RAF inhibitor resistance is mediated by dimerization of aberrantly spliced
1187 BRAF(V600E). *Nature* **480** 387-390.
1188 Proud CG 2013 mTORC1 regulates the efficiency and cellular capacity for protein synthesis.
1189 *Biochem Soc Trans* **41** 923-926.

1190 Pyronnet S, Imataka H, Gingras AC, Fukunaga R, Hunter T & Sonenberg N 1999 Human
1191 eukaryotic translation initiation factor 4G (eIF4G) recruits mnk1 to phosphorylate eIF4E. *EMBO*
1192 *J* **18** 270-279.
1193 Ramanathan A & Schreiber SL 2009 Direct control of mitochondrial function by mTOR. *Proc*
1194 *Natl Acad Sci U S A* **106** 22229-22232.
1195 Raught B, Peiretti F, Gingras AC, Livingstone M, Shahbazian D, Mayeur GL, Polakiewicz RD,
1196 Sonenberg N & Hershey JW 2004 Phosphorylation of eucaryotic translation initiation factor 4B
1197 Ser422 is modulated by S6 kinases. *EMBO J* **23** 1761-1769.
1198 Reitzer LJ, Wice BM & Kennell D 1979 Evidence that glutamine, not sugar, is the major energy
1199 source for cultured HeLa cells. *J Biol Chem* **254** 2669-2676.
1200 Robichaud N, del Rincon SV, Huor B, Alain T, Petruccelli LA, Hearnden J, Goncalves C,
1201 Grotegut S, Spruck CH, Furic L, et al. 2015 Phosphorylation of eIF4E promotes EMT and
1202 metastasis via translational control of SNAIL and MMP-3. *Oncogene* **34** 2032-2042.
1203 Robitaille AM, Christen S, Shimobayashi M, Cornu M, Fava LL, Moes S, Prescianotto-
1204 Baschong C, Sauer U, Jenoe P & Hall MN 2013 Quantitative phosphoproteomics reveal
1205 mTORC1 activates de novo pyrimidine synthesis. *Science* **339** 1320-1323.
1206 Rodrik-Outmezguine VS, Okaniwa M, Yao Z, Novotny CJ, McWhirter C, Banaji A, Won H,
1207 Wong W, Berger M, de Stanchina E, et al. 2016 Overcoming mTOR resistance mutations with a
1208 new-generation mTOR inhibitor. *Nature* **534** 272-276.
1209 Rogers GW, Jr., Richter NJ & Merrick WC 1999 Biochemical and kinetic characterization of the
1210 RNA helicase activity of eukaryotic initiation factor 4A. *J Biol Chem* **274** 12236-12244.
1211 Rolfe DF & Brown GC 1997 Cellular energy utilization and molecular origin of standard
1212 metabolic rate in mammals. *Physiol Rev* **77** 731-758.
1213 Roux PP, Ballif BA, Anjum R, Gygi SP & Blenis J 2004 Tumor-promoting phorbol esters and
1214 activated Ras inactivate the tuberous sclerosis tumor suppressor complex via p90 ribosomal S6
1215 kinase. *Proc Natl Acad Sci U S A* **101** 13489-13494.
1216 Roux PP & Topisirovic I 2012 Regulation of mRNA translation by signaling pathways. *Cold*
1217 *Spring Harb Perspect Biol* **4**.
1218 Roux PP & Topisirovic I 2018 Signaling pathways involved in the regulation of mRNA
1219 translation. *Mol Cell Biol*.
1220 Ruggero D 2013 Translational control in cancer etiology. *Cold Spring Harb Perspect Biol* **5**.
1221 Ruggero D & Sonenberg N 2005 The Akt of translational control. *Oncogene* **24** 7426-7434.
1222 Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, Nir T, Dor Y, Zisman P & Meyuhas
1223 O 2005 Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose
1224 homeostasis. *Genes Dev* **19** 2199-2211.
1225 Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S & Sabatini DM 2010 Ragulator-Rag
1226 complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino
1227 acids. *Cell* **141** 290-303.
1228 Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L & Sabatini DM
1229 2008 The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* **320**
1230 1496-1501.
1231 Sancak Y, Thoreen CC, Peterson TR, Lindquist RA, Kang SA, Spooner E, Carr SA & Sabatini
1232 DM 2007 PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase. *Mol Cell* **25**
1233 903-915.

1234 Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL & Sabatini
1235 DM 2006 Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell*
1236 **22** 159-168.
1237 Saxton RA & Sabatini DM 2017 mTOR Signaling in Growth, Metabolism, and Disease. *Cell*
1238 **169** 361-371.
1239 Scheper GC, van Kollenburg B, Hu J, Luo Y, Goss DJ & Proud CG 2002 Phosphorylation of
1240 eukaryotic initiation factor 4E markedly reduces its affinity for capped mRNA. *J Biol Chem* **277**
1241 3303-3309.
1242 Schieke SM, Phillips D, McCoy JP, Jr., Aponte AM, Shen RF, Balaban RS & Finkel T 2006 The
1243 mammalian target of rapamycin (mTOR) pathway regulates mitochondrial oxygen consumption
1244 and oxidative capacity. *J Biol Chem* **281** 27643-27652.
1245 Schlessinger J 2002 Ligand-induced, receptor-mediated dimerization and activation of EGF
1246 receptor. *Cell* **110** 669-672.
1247 Schwanhausser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W & Selbach M 2011
1248 Global quantification of mammalian gene expression control. *Nature* **473** 337-342.
1249 Semenza GL 2000 HIF-1: mediator of physiological and pathophysiological responses to
1250 hypoxia. *J Appl Physiol (1985)* **88** 1474-1480.
1251 Semenza GL 2007 Hypoxia-inducible factor 1 (HIF-1) pathway. *Sci STKE* **2007** cm8.
1252 Shaw RJ 2009 LKB1 and AMP-activated protein kinase control of mTOR signalling and growth.
1253 *Acta Physiol (Oxf)* **196** 65-80.
1254 Shaw RJ, Bardeesy N, Manning BD, Lopez L, Kosmatka M, DePinho RA & Cantley LC 2004a
1255 The LKB1 tumor suppressor negatively regulates mTOR signaling. *Cancer Cell* **6** 91-99.
1256 Shaw RJ, Kosmatka M, Bardeesy N, Hurley RL, Witters LA, DePinho RA & Cantley LC 2004b
1257 The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates
1258 apoptosis in response to energy stress. *Proc Natl Acad Sci U S A* **101** 3329-3335.
1259 Shestov AA, Liu X, Ser Z, Cluntun AA, Hung YP, Huang L, Kim D, Le A, Yellen G, Albeck
1260 JG, et al. 2014 Quantitative determinants of aerobic glycolysis identify flux through the enzyme
1261 GAPDH as a limiting step. *Elife* **3**.
1262 Shim H, Dolde C, Lewis BC, Wu CS, Dang G, Jungmann RA, Dalla-Favera R & Dang CV 1997
1263 c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. *Proc Natl*
1264 *Acad Sci U S A* **94** 6658-6663.
1265 Shimobayashi M & Hall MN 2014 Making new contacts: the mTOR network in metabolism and
1266 signalling crosstalk. *Nat Rev Mol Cell Biol* **15** 155-162.
1267 Shuvalov O, Petukhov A, Daks A, Fedorova O, Vasileva E & Barlev NA 2017 One-carbon
1268 metabolism and nucleotide biosynthesis as attractive targets for anticancer therapy. *Oncotarget* **8**
1269 23955-23977.
1270 Siekierka JJ, Hung SH, Poe M, Lin CS & Sigal NH 1989 A cytosolic binding protein for the
1271 immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from
1272 cyclophilin. *Nature* **341** 755-757.
1273 Silvera D, Formenti SC & Schneider RJ 2010 Translational control in cancer. *Nat Rev Cancer* **10**
1274 254-266.
1275 Slepenkov SV, Darzynkiewicz E & Rhoads RE 2006 Stopped-flow kinetic analysis of eIF4E and
1276 phosphorylated eIF4E binding to cap analogs and capped oligoribonucleotides: evidence for a
1277 one-step binding mechanism. *J Biol Chem* **281** 14927-14938.
1278 Slomovitz BM & Coleman RL 2012 The PI3K/AKT/mTOR pathway as a therapeutic target in
1279 endometrial cancer. *Clin Cancer Res* **18** 5856-5864.

1280 Smallbone K, Gavaghan DJ, Gatenby RA & Maini PK 2005 The role of acidity in solid tumour
1281 growth and invasion. *J Theor Biol* **235** 476-484.
1282 Sonenberg N & Hinnebusch AG 2009 Regulation of translation initiation in eukaryotes:
1283 mechanisms and biological targets. *Cell* **136** 731-745.
1284 Soukas AA, Kane EA, Carr CE, Melo JA & Ruvkun G 2009 Rictor/TORC2 regulates fat
1285 metabolism, feeding, growth, and life span in Caenorhabditis elegans. *Genes Dev* **23** 496-511.
1286 Spriggs KA, Bushell M & Willis AE 2010 Translational regulation of gene expression during
1287 conditions of cell stress. *Mol Cell* **40** 228-237.
1288 Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, Ruland J, Penninger
1289 JM, Siderovski DP & Mak TW 1998 Negative regulation of PKB/Akt-dependent cell survival by
1290 the tumor suppressor PTEN. *Cell* **95** 29-39.
1291 Stark H, Rodnina MV, Wieden HJ, van Heel M & Wintermeyer W 2000 Large-scale movement
1292 of elongation factor G and extensive conformational change of the ribosome during
1293 translocation. *Cell* **100** 301-309.
1294 Starosta AL, Lassak J, Jung K & Wilson DN 2014 The bacterial translation stress response.
1295 *FEMS Microbiol Rev* **38** 1172-1201.
1296 Stine ZE, Walton ZE, Altman BJ, Hsieh AL & Dang CV 2015 MYC, Metabolism, and Cancer.
1297 *Cancer Discov* **5** 1024-1039.
1298 Svitkin YV, Pause A, Haghighat A, Pyronnet S, Witherell G, Belsham GJ & Sonenberg N 2001
1299 The requirement for eukaryotic initiation factor 4A (elF4A) in translation is in direct proportion
1300 to the degree of mRNA 5' secondary structure. *RNA* **7** 382-394.
1301 Taylor DJ, Nilsson J, Merrill AR, Andersen GR, Nissen P & Frank J 2007 Structures of modified
1302 eEF2 80S ribosome complexes reveal the role of GTP hydrolysis in translocation. *EMBO J* **26**
1303 2421-2431.
1304 Tcherkezian J, Cargnello M, Romeo Y, Huttlin EL, Lavoie G, Gygi SP & Roux PP 2014
1305 Proteomic analysis of cap-dependent translation identifies LARP1 as a key regulator of 5'TOP
1306 mRNA translation. *Genes Dev* **28** 357-371.
1307 Thoreen CC, Chantranupong L, Keys HR, Wang T, Gray NS & Sabatini DM 2012 A unifying
1308 model for mTORC1-mediated regulation of mRNA translation. *Nature* **485** 109-113.
1309 Topisirovic I, Ruiz-Gutierrez M & Borden KL 2004 Phosphorylation of the eukaryotic
1310 translation initiation factor eIF4E contributes to its transformation and mRNA transport
1311 activities. *Cancer Res* **64** 8639-8642.
1312 Topisirovic I & Sonenberg N 2011 mRNA translation and energy metabolism in cancer: the role
1313 of the MAPK and mTORC1 pathways. *Cold Spring Harb Symp Quant Biol* **76** 355-367.
1314 Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR,
1315 Kozma SC, Auwerx J, et al. 2004 Absence of S6K1 protects against age- and diet-induced
1316 obesity while enhancing insulin sensitivity. *Nature* **431** 200-205.
1317 van Riggelen J, Yetil A & Felsher DW 2010 MYC as a regulator of ribosome biogenesis and
1318 protein synthesis. *Nat Rev Cancer* **10** 301-309.
1319 Vander Heiden MG, Cantley LC & Thompson CB 2009 Understanding the Warburg effect: the
1320 metabolic requirements of cell proliferation. *Science* **324** 1029-1033.
1321 Wan X, Harkavy B, Shen N, Grohar P & Helman LJ 2007 Rapamycin induces feedback
1322 activation of Akt signaling through an IGF-1R-dependent mechanism. *Oncogene* **26** 1932-1940.
1323 Wang W, Fridman A, Blackledge W, Connelly S, Wilson IA, Pilz RB & Boss GR 2009 The
1324 phosphatidylinositol 3-kinase/akt cassette regulates purine nucleotide synthesis. *J Biol Chem* **284**
1325 3521-3528.

Wang X, Li W, Williams M, Terada N, Alessi DR & Proud CG 2001 Regulation of elongation factor 2 kinase by p90(RSK1) and p70 S6 kinase. *EMBO J* **20** 4370-4379.
Wang X & Proud CG 2006 The mTOR pathway in the control of protein synthesis. *Physiology (Bethesda)* **21** 362-369.
Warburg O 1925 The Metabolism of Carcinoma Cells. *The Journal of Cancer Research* **9** 148-163.
Warburg O 1956 On the origin of cancer cells. *Science* **123** 309-314.
Ward PS & Thompson CB 2012 Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. *Cancer Cell* **21** 297-308.
Waskiewicz AJ, Flynn A, Proud CG & Cooper JA 1997 Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2. *EMBO J* **16** 1909-1920.
Waskiewicz AJ, Johnson JC, Penn B, Mahalingam M, Kimball SR & Cooper JA 1999 Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 in vivo. *Mol Cell Biol* **19** 1871-1880.
Wendel HG, Silva RL, Malina A, Mills JR, Zhu H, Ueda T, Watanabe-Fukunaga R, Fukunaga R, Teruya-Feldstein J, Pelletier J, et al. 2007 Dissecting eIF4E action in tumorigenesis. *Genes Dev* **21** 3232-3237.
Wenger RH 2000 Mammalian oxygen sensing, signalling and gene regulation. *J Exp Biol* **203** 1253-1263.
Wheaton WW, Weinberg SE, Hamanaka RB, Soberanes S, Sullivan LB, Anso E, Glasauer A, Dufour E, Mutlu GM, Budigner GS, et al. 2014 Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. *Elife* **3** e02242.
Whiteman EL, Cho H & Birnbaum MJ 2002 Role of Akt/protein kinase B in metabolism. *Trends Endocrinol Metab* **13** 444-451.
Winkler BS, DeSantis N & Solomon F 1986 Multiple NADPH-producing pathways control glutathione (GSH) content in retina. *Exp Eye Res* **43** 829-847.
Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB, et al. 2008 Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. *Proc Natl Acad Sci U S A* **105** 18782-18787.
Wise DR & Thompson CB 2010 Glutamine addiction: a new therapeutic target in cancer. *Trends Biochem Sci* **35** 427-433.
Wolfson RL, Chantranupong L, Wyant GA, Gu X, Orozco JM, Shen K, Condon KJ, Petri S, Kedir J, Scaria SM, et al. 2017 KICSTOR recruits GATOR1 to the lysosome and is necessary for nutrients to regulate mTORC1. *Nature* **543** 438-442.
Yang H, Rudge DG, Koos JD, Vaidialingam B, Yang HJ & Pavletich NP 2013 mTOR kinase structure, mechanism and regulation. *Nature* **497** 217-223.
Yao R & Cooper GM 1995 Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science* **267** 2003-2006.
Yecies JL & Manning BD 2011 Transcriptional control of cellular metabolism by mTOR signaling. *Cancer Res* **71** 2815-2820.
Young K, Minchom A & Larkin J 2012 BRIM-1, -2 and -3 trials: improved survival with vemurafenib in metastatic melanoma patients with a BRAFV600E mutation. *Future Oncology* **8** 499-507.

Yuan P, Ito K, Perez-Lorenzo R, Del Guzzo C, Lee JH, Shen CH, Bosenberg MW, McMahon M, Cantley LC & Zheng B 2013 Phenformin enhances the therapeutic benefit of BRAF(V600E) inhibition in melanoma. *Proc Natl Acad Sci U S A* **110** 18226-18231.

Zech R, Kiontke S, Mueller U, Oeckinghaus A & Kummel D 2016 Structure of the Tuberous Sclerosis Complex 2 (TSC2) N Terminus Provides Insight into Complex Assembly and Tuberous Sclerosis Pathogenesis. *J Biol Chem* **291** 20008-20020.

Zhan Y, Dahabieh MS, Rajakumar A, Dobocan MC, M'Boutchou MN, Goncalves C, Lucy SL, Pettersson F, Topisirovic I, van Kempen L, et al. 2015 The role of eIF4E in response and acquired resistance to vemurafenib in melanoma. *J Invest Dermatol* **135** 1368-1376.

Zhang CS, Hawley SA, Zong Y, Li M, Wang Z, Gray A, Ma T, Cui J, Feng JW, Zhu M, et al. 2017 Fructose-1,6-bisphosphate and aldolase mediate glucose sensing by AMPK. *Nature* **548** 112-116.

Zhang HH, Huang J, Duvel K, Boback B, Wu S, Squillace RM, Wu CL & Manning BD 2009 Insulin stimulates adipogenesis through the Akt-TSC2-mTORC1 pathway. *PLoS One* **4** e6189.

Zhao F, Mancuso A, Bui TV, Tong X, Gruber JJ, Swider CR, Sanchez PV, Lum JJ, Sayed N, Melo JV, et al. 2010 Imatinib resistance associated with BCR-ABL upregulation is dependent on HIF-1alpha-induced metabolic reprograming. *Oncogene* **29** 2962-2972.

Zhou H & Huang S 2011 Role of mTOR signaling in tumor cell motility, invasion and metastasis. *Curr Protein Pept Sci* **12** 30-42.

Zindy P, Berge Y, Allal B, Filleron T, Pierredon S, Cammas A, Beck S, Mhamdi L, Fan L, Favre G, et al. 2011 Formation of the eIF4F translation-initiation complex determines sensitivity to anticancer drugs targeting the EGFR and HER2 receptors. *Cancer Res* **71** 4068-4073.

Zoncu R, Bar-Peled L, Efeyan A, Wang S, Sancak Y & Sabatini DM 2011 mTORC1 senses lysosomal amino acids through an inside-out mechanism that requires the vacuolar H(+)-ATPase. *Science* **334** 678-683.

Figure legends

Figure 1. **Schematic representation of signaling pathways upstream of MTORC1.** MTOR exists in two functionally and structurally distinct complexes: MTORC1 and MTORC2. MTORC1 is activated by hormones (e.g. insulin) or growth factors (e.g. EGF, FGF) via receptor tyrosine kinases. This sets off a signaling cascade leading to the activation of PI3K which inactivates TSC2 via AKT. In addition, TSC2 is inhibited by the MAPK/RSK pathway, and activated by DDIT4 and AMPK in response to hypoxia, and nutrient/energy depletion, respectively. TSC inactivation leads to MTORC1 activation, though the intermediary of RHEB. Amino acids stimulate LAMTOR, a GEF for the heterodimeric RRAG complex, which in turn activates MTORC1. Pharmacological inhibitors that potentially impact on the cross-talk between mTOR-dependent translational and metabolic programs are indicated. Further explanations are provided in the text.

Abbreviations: MTOR, mechanistic target of rapamycin kinase; MTORC1, mechanistic target of rapamycin complex 1; MTORC2, mechanistic target of rapamycin complex 2; EGF, epidermal growth factor; FGF, fibroblast growth factor; PI3K, phosphoinositide 3-kinase; TSC2, tuberous sclerosis complex 2; MAPK, mitogen-activated protein kinase; RSK, p90 ribosomal S6 kinase; DDIT4, DNA damage inducible transcript 4; AKT, protein kinase B; AMPK, AMP-activated protein kinase; RHEB, ras homolog, MTORC1 binding; LAMTOR, late endosomal and lysosomal adaptor and MAPK (mitogen-activated protein kinase) and MTOR (mechanistic target of rapamycin) activator; GEF, guanine nucleotide exchange factor; RRAG, ras-related GTP-binding protein.

Figure 2. **Schematic representation of effectors downstream of MTORC1.** MTORC1 controls various metabolic processes via transcriptional and/or translational regulation. MTORC1 stimulates nucleotide synthesis (via ATF4), pentose phosphate pathway and lipid

biosynthesis (via SREBP1), adipogenesis (via PPARG), glutamine metabolism (via ATF2 and MYC) and mitochondrial biogenesis (via PPARGC1A and YY1). The MTORC1/RPS6KB axis stimulates SREBP1 and CAD, which are essential for lipid and nucleotide biosynthesis, respectively. MTORC1 also stimulates lipid synthesis by controlling the nuclear localization of LPIN1, a negative regulator of SREBP1. In addition, MTORC1 phosphorylates LPIN1, facilitating its role as a co-activator for PPARG during adipogenesis. To date, the best characterized mediators of the effects of MTOR on protein synthesis are EIF4EBPs and RPS6KBs. MTOR stimulates the EIF4F complex assembly (comprised of EIF4E, EIF4G and EIF4A), by inactivating EIF4EBPs. In turn, RPS6KBs phosphorylate components of the translational machinery (PDCD4, RPS6, EIF4B and EEF2K). In respect to cancer energetics, the MTORC1/EIF4EBP/EIF4F axis regulates translation of mRNAs encoding mitochondrial factors (e.g. TFAM, ATP5O), central metabolic regulators (MYC and HIF1A) and enzymes involved in non-essential amino acid synthesis (PHGDH, PSAT1, PC and ASNS). The effects of EIF4A inhibitors and SBI-756 on MTOR-dependent translational and metabolic programs in explained within the text.

Abbreviations: ATF4, activating transcription factor 4; SREBP1, sterol regulatory element-binding transcription factor 1; PPARG, peroxisome proliferator-activated receptor gamma; ATF2, activating transcription factor 2; PPARGC1A, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; YY1, Yin Yang 1; RPS6KB, ribosomal protein S6 kinase; CAD, carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase; LPIN1, lipin 1; EIF4EBP, eukaryotic initiation factor 4E-binding protein; EIF, eukaryotic initiation factor; PDCD4, programmed cell death 4; RPS6, ribosomal protein S6; EEF2K, eukaryotic elongation factor 2 kinase; TFAM, transcription factor A, mitochondrial; ATP5O, ATP synthase subunit O; HIF1A, hypoxia-inducible factor 1A; PRPS2, phosphoribosyl pyrophosphate synthetase 2; PHDH, phosphoglycerate

dehydrogenase; PSAT1, phosphoserine aminotransferase1; PC, pyruvate carboxylase; ASNS, asparagine synthetase.

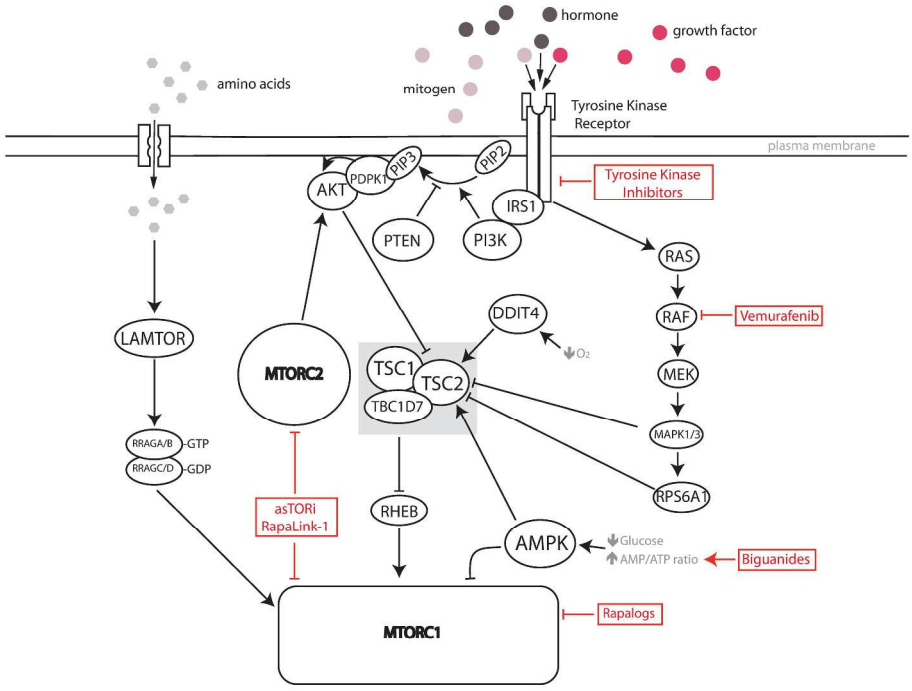

Figure 1. Schematic representation of signaling pathways upstream of MTORC1.
MTOR exists in two functionally and structurally distinct complexes: MTORC1 and MTORC2. MTORC1 is activated by hormones (e.g. insulin) or growth factors (e.g. EGF, FGF) via receptor tyrosine kinases. This sets off a signaling cascade leading to the activation of PI3K which inactivates TSC2 via AKT. In addition, TSC2 is inhibited by the MAPK/RSK pathway, and activated by DDIT4 and AMPK in response to hypoxia, and nutrient/energy depletion, respectively. TSC inactivation leads to MTORC1 activation, though the intermediary of RHEB. Amino acids stimulate LAMTOR, a GEF for the heterodimeric RRAG complex, which in turn activates MTORC1. Pharmacological inhibitors that potentially impact on the cross-talk between mTOR-dependent translational and metabolic programs are indicated. Further explanations are provided in the text.
Abbreviations: MTOR, mechanistic target of rapamycin kinase; MTORC1, mechanistic target of rapamycin complex 1; MTORC2, mechanistic target of rapamycin complex 2; EGF, epidermal growth factor; FGF, fibroblast growth factor; PI3K, phosphoinositide 3-kinase; TSC2, tuberous sclerosis complex 2; MAPK, mitogen-activated protein kinase; RSK, p90 ribosomal S6 kinase; DDIT4, DNA damage inducible transcript

4; AKT, protein kinase B; AMPK, AMP-activated protein kinase; RHEB, ras homolog, MTORC1 binding; LAMTOR, late endosomal and lysosomal adaptor and MAPK (mitogen-activated protein kinase) and MTOR (mechanistic target of rapamycin) activator; GEF, guanine nucleotide exchange factor; RRAG, ras-related GTP-binding protein.

279x361mm (300 x 300 DPI)

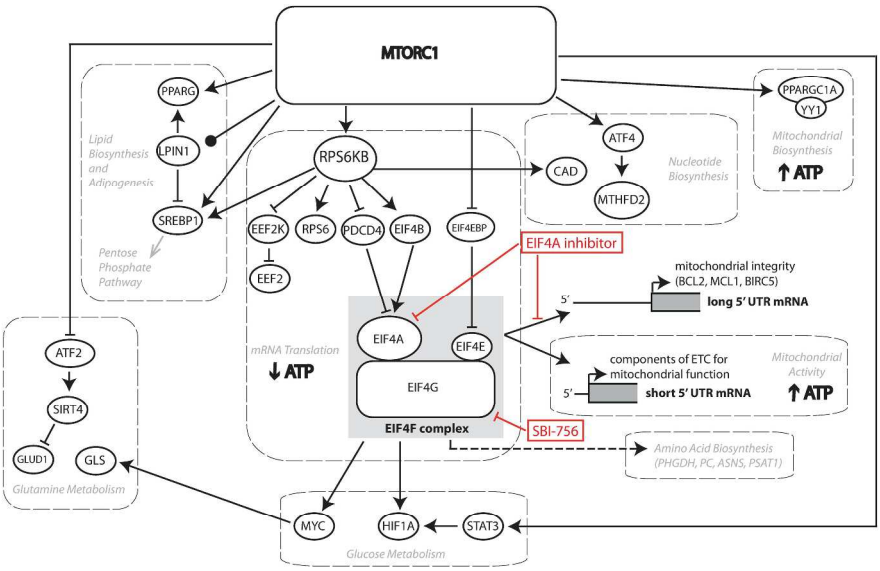

Figure 2. Schematic representation of effectors downstream of MTORC1.
MTORC1 controls various metabolic processes via transcriptional and/or translational regulation. MTORC1 stimulates nucleotide synthesis (via ATF4), pentose phosphate pathway and lipid biosynthesis (via SREBP1), adipogenesis (via PPARG), glutamine metabolism (via ATF2 and MYC) and mitochondrial biogenesis (via PPARGC1A and YY1). The MTORC1/RPS6KB axis stimulates SREBP1 and CAD, which are essential for lipid and nucleotide biosynthesis, respectively. MTORC1 also stimulates lipid synthesis by controlling the nuclear localization of LPIN1, a negative regulator of SREBP1. In addition, MTORC1 phosphorylates LPIN1, facilitating its role as a co-activator for PPARG during adipogenesis. To date, the best characterized mediators of the effects of MTOR on protein synthesis are EIF4EBPs and RPS6KBs. MTOR stimulates the EIF4F complex assembly (comprised of EIF4E, EIF4G and EIF4A), by inactivating EIF4EBPs. In turn, RPS6KBs phosphorylate components of the translational machinery (PDCD4, RPS6, EIF4B and EEF2K). In respect to cancer energetics, the MTORC1/EIF4EBP/EIF4F axis regulates translation of mRNAs encoding mitochondrial factors (e.g. TFAM, ATP5O), central metabolic regulators (MYC and HIF1A) and enzymes

involved in non-essential amino acid synthesis (PHGDH, PSAT1, PC and ASNS). The effects of EIF4A inhibitors and SBI-756 on MTOR-dependent translational and metabolic programs in explained within the text.

Abbreviations: ATF4, activating transcription factor 4; SREBP1, sterol regulatory element-binding transcription factor 1; PPARG, peroxisome proliferator-activated receptor gamma; ATF2, activating transcription factor 2; PPARGC1A, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; YY1, Yin Yang 1; RPS6KB, ribosomal protein S6 kinase; CAD, carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase; LPIN1, lipin 1; EIF4EBP, eukaryotic initiation factor 4E-binding protein; EIF, eukaryotic initiation factor; PDCD4, programmed cell death 4; RPS6, ribosomal protein S6; EEF2K, eukaryotic elongation factor 2 kinase; TFAM, transcription factor A, mitochondrial; ATP5O, ATP synthase subunit O; HIF1A, hypoxia-inducible factor 1A; PRPS2, phosphoribosyl pyrophosphate synthetase 2; PHDH, phosphoglycerate dehydrogenase; PSAT1, phosphoserine aminotransferase1; PC, pyruvate carboxylase; ASNS, asparagine synthetase.

279x361mm (300 x 300 DPI)