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

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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.

Key Words
- protein synthesis
- metabolism
- cancer
- MTOR
- oncogenic kinases

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 (Johnson et al. 1976, Kevil et al. 1996, Larsson et al. 2006, Larsson et al. 2007, Hanahan & Weinberg 2011, Topisirovic & 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 (Giuseppe 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 tumor 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 favors the development of drug resistance, angiogenesis, survival, proliferation and metastasis. For instance, high levels of eukaryotic translation initiator factor 4E (eIF4E (proteins written in bold are represented in the figures)) 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 & Hinnebusch 2009), which are required for adaptation to stress (Spriggs et al. 2010,
Guan et al. 2017). The importance of gene expression regulation at the translational level is evident as steady-state mRNA levels are thought to have 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 & Brand 1995, Rolfe & 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 & 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 & 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 (Vander Heiden et al. 2009, Locasale & Cantley 2011, Shestov et al. 2014). 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 & 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 (Jessani et al. 2004, Hsu & Sabatini 2008, Dang et al. 2011).

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.

### P13K/AKT – mechanisms of activation and regulation of metabolic functions

The phosphatidylinositol-4,5-biphosphate 3-kinase (P13K)/AKT signaling pathway regulates many essential processes including cell growth, mRNA translation, proliferation, survival, apoptosis and metabolism (Yao & Cooper 1995, Kauffmann-Zeh et al. 1997, Laplante & Sabatini 2009b). Aberrant signaling via this pathway has been implicated in pathological conditions including diabetes and cancer, whereby its hyperactivation in general is tumor promoting (Laplante & Sabatini 2012, Porta et al. 2014).

The P13K/AKT signaling cascade is activated when receptor tyrosine kinases such as insulin receptors are bound by their ligands, including insulin and/or growth factors (Ruggero & Sonenberg 2005) (Fig. 1). The extracellular binding of the ligands results in intracellular autophosphorylation of tyrosine residues on the receptors (Schlessinger 2002, Lemmon & Schlessinger 2010). The phosphorylated tyrosine residues recruit P13K to the membrane (Dommek et al. 1992). At the membrane, P13K phosphorylates phosphatidylinositol-4,5-biphosphate (PIP$_2$) to produce phosphatidylinositol-3,4,5-triphosphate (PIP$_3$) (Fig. 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 PDK1 (3-phosphoinositide-dependent protein kinase 1) (Fig. 1) (Alessi et al. 1997, Fresno Vara et al. 2004).

AKT is a serine/threonine protein kinase that regulates cell survival, growth and proliferation (Wan et al. 2007, Myers & Cantley 2010). AKT carries out its functions...
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through various downstream effectors including MTOR (Slomovitz & 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 (Fig. 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 (Whiteman et al. 2002, Elstrom et al. 2004). 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/fructose-2,6-biphosphatase (PFKFB) (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 & 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).

**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 (Wang & Proud 2006, Laplante & Sabatini 2009a). It integrates extracellular and intracellular signals emanating from environmental cues, nutrient availability and cellular energetic status (Liu et al. 2009,

![Diagram 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 DDI4 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. 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; DDI4, 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.

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Regulation of MTORC1 activity

Upon activation of the pathway, AKT phosphorylates TSC complex subunit 2 (TSC2), which heterotrimerize with TSC complex subunit 1 (TSC1) and TBC1 domain family member 7 (TBC1D7) (Zech et al. 2016) (Fig. 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 (Fig. 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 (Fig. 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 (Fig. 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 (Roux et al. 2004, Memmott & Dennis 2009) (Fig. 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) (Fig. 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 ‘MTORC1 and the regulation of mRNA translation’ section.

MTORC1 and the regulation of mRNA translation

mRNA translation occurs in four 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 & 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 & Hinnebusch 2009). The initiation phase of mRNA cap-dependent translation involves the assembly of a 43S PIC, 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\_Met (Hinnebusch 2014). The EIF4F complex contains three subunits: EIF4E (mRNA cap-binding subunit), EIF4A ( DEAD box RNA helicase) and EIF4G1 (scaffolding protein) (Fig. 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 & Sonenberg 2012), the mRNA codons dictate the sequence of specific tRNAs that go through the acylation-peptidyl-exit 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 & Sonenberg 2012). The bound GTP is
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hydrolyzed when the anticodon of the incoming tRNA is matched against the corresponding mRNA codon (Agirrezabala & Frank 2009). This process results in the formation of a peptide bond (Mohr & Sonenberg 2012). The activity of EIF1A1 is regulated by EIF1B2 (a guanine exchange factor (GEF)) (Mohr & Sonenberg 2012). EIF2 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 & 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 (Wang et al. 2001, Hsieh et al. 2012, Thoreen et al. 2012, Proud 2013). 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 (Fig. 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 EIF4G1 (Fig. 2). Upon EIF4EBPs phosphorylation, EIF4E is released to form the active EIF4F complex (Sonenberg & 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, Waskiewicz et al. 1997, Knauf et al. 2001). 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 (Schepet 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. SNAI1 (snail family transcriptional repressor 1), MMPs (matrix metalloproteinases)) and cytokines (Furic et al. 2010, Robichaud et al. 2015). Overall, EIF4F assembly is required for recruiting mRNAs to the ribosome, which is an essential step in initiating cap-dependent mRNA translation (Pause et al. 1994, Gingras et al. 1999).

MTORC1 also controls protein synthesis through the phosphorylation and activation of RPS6KBs (Roux & 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) (Holz et al. 2005, Dorrello et al. 2006, Chauvin et al. 2014) (Fig. 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 (Dorrello et al. 2006, Dennis et al. 2012). 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 & Proud 2004, Browne et al. 2004). mRNA translation-related processes that 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 & 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 & 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 andpoly (A)-binding proteins (PABPs), and their translation is dramatically suppressed by MTOR inhibitors (Meyuhas & Dreazen 2009, Hsieh et al. 2012). Initially, it was proposed that the RPS6KBs/RPS6 axis mediated the regulatory effects of MTOR on the translation of TOP mRNAs (Jefferies et al. 1994, 1997). Subsequently, it was however found that there was no difference in the translation of TOP mRNAs when cells deficient in RPS6KB and expressing non-phosphorylatable RPS6 (i.e. RPS6 knock-in) were compared to WT 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 (Tcherkezian et al. 2014, Fonseca et al. 2015, Hong et al. 2017, Philippe et al. 2018). Furthermore, the context in which translation takes place is known to affect the translation of TOP mRNAs.
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MTORC1 stimulates glycolysis in part through the translational regulation of transcription factors such as MYC and hypoxia-inducible factor 1A (HIF1A) (Gordan et al. 2007, Duvel et al. 2010). In some cell types, MTORC1 regulates HIF1A translationally via EIF4EBP1 and RPS6KB1 (Dodd et al. 2015) (Fig. 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 (Semenza 2000, Wenger 2000, Keith et al. 2011). 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 (Shim et al. 1997, Osthus et al. 2000, Kim et al. 2007).

Glutamine is one of the most readily available non-essential 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 & Thompson 2010). Activated MTORC1 stimulates glutaminolysis, whereby glutamine is converted to glutamate by glutaminase (GLS) (Fig. 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) (Fig. 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 \textit{EIF4A} (Csibi et al. 2014) (Fig. 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.

**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 & Sabatini 2017). In mammals, heterodimeric RRAG (Ras-related GTP binding) GTPases regulate **MTORC1** signaling in response to amino acid levels (Fig. 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 composed 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 toward RRAGs (Sancak et al. 2010, Bar-Peled et al. 2012). The presence of amino acids stimulates RRAG heterodimers whereby RRAGC/D and RRAGA/B are GDP and GTP bound, respectively (Sancak et al. 2008) (Fig. 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 MOIS (meiosis regulator for oocyte development), WDR24 (WD repeat domain 24), WDR59, SEH1L (SEH1 like neureoporin), 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 non-essential amino acids by stimulating glycolysis, TCA cycle and pentose phosphate pathways (Duvel et al. 2010, Yecies & Manning 2011), which provide key metabolites necessary for amino acids synthesis (Duvel et al. 2010, Yecies & 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 (Sarbassov et al. 2006, Lamming et al. 2012). 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 unwinding of the structured 5'UTR of **MYC** by \textit{EIF4A} (Csibi et al. 2014) (Fig. 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.
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.

MTOR stimulates lipid synthesis

Rapidly dividing malignant cells require increased synthesis of lipids, which are the main components of plasma and organelle membranes (Menendez & 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 (SREBF1 and SREBF2), 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 (Porstmann et al. 2008, Duvel et al. 2010, Li et al. 2016) (Fig. 2). Consistently, rapamycin downregulates the expression of SREBF 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 SREBF1 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 SREBF1 activity (Peterson et al. 2011). MTORC1 phosphorylates LPIN1, which prevents its translocation to the nucleus and thereby prevents the LPIN1-dependent suppression of SREBP activity (Peterson et al. 2011) (Fig. 2). Finally, MTOR can activate SREBF1 by phosphorylating CREB regulated transcription coactivator 2 (CRTC2) (Han et al. 2015), which attenuates CRTC2 inhibitory effects on the processing of SREBF1 (Han et al. 2015).

In addition to SREBPs, MTORC1 influences lipid metabolism by upregulating the activity of peroxisome proliferator-activated receptor gamma (PPARG) (Kim & Chen 2004) (Fig. 2). Hyperactivation of the MTORC1 pathway stimulates PPARG-dependent adipogenesis (Zhang et al. 2009), while rapamycin leads to the reduction of both mRNA and protein levels of PPARG and the inhibition of adipogenesis (Cho et al. 2004, Kim & Chen 2004). There is evidence showing that MTORC1 mediates its effects on the regulation of PPARG via EIF4EBPs and RPS6KB1 (Um et al. 2004, Le Bacquer et al. 2007). 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) (Fig. 2). On the basis of these findings, MTORC1 regulates lipid synthesis chiefly by perturbing activity of SREBPs and PPARG.

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 (Horecker et al. 1951, Glaser & Brown 1955, Dickens & Williamson 1956). 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 (Fig. 2) (Duvel et al. 2010), while 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) (Fig. 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) (Fig. 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 an 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.

**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 & Sonenberg 2011). To this end, malignant cells must meet the heightened energy requirement caused by elevated energy consumption by the protein synthesis apparatus (Ward & Thompson 2012, Ruggero 2013). 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 an 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) (Fig. 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) (Fig. 2). Finally, the MTORC1/EIF4EBP axis has been shown to regulate mitochondrial dynamics by modulating translation of mitochondrial fission process 1 (MTPF1) (Morita et al. 2017).

In addition to translational regulation, MTORC1 regulates the transcription of mitochondrial genes via PPARG coactivator 1 alpha (PPARGC1A) (Fig. 2) (Cunningham et al. 2007). The inhibition of MTORC1 by rapamycin decreased the expression of mitochondrial transcriptional regulators PPARGC1A, estrogen-related receptor alpha (ESRRA) and nuclear respiratory factors, 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 (Fig. 2) (Cunningham et al. 2007).

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

**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.

**Role of MTORC2 in metabolic regulation**

MTORC2 is known to regulate cell survival, metabolism, cytoskeletal organization and cell migration (Oh & 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 hyperlipidemia (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.

**Therapeutic implications of the cross-talk between translometabolome**

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, PPP and sterol and lipid biosynthesis (Peng et al. 2002, Schieke et al. 2006, Cunningham et al. 2007, Ramanathan & Schreiber 2009, Duvel et al. 2010). At the organismal level, rapamycin treatment results 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 analogs (rapalogs; Fig. 1) are FDA approved for the treatment of renal cell carcinomas, mantle cell lymphomas and pancreatic neuroendocrine tumors (Li et al. 2014a). However, the efficacy in the clinic is not as good as initially hoped (Fasolo & Sessa 2008). This can been justified in part by the activation of AKT via the suppression of the RPS6KB1-IRS1-PI3K-AKT regulatory feedback, as well as by rapamycin’s inability to inhibit certain MTORC1 outputs including phosphorylation of EIF4EBPs (Dowling et al. 2010, Faes et al. 2017). To this end, more efficient means to target MTOR were developed, including active site MTOR inhibitors (aSTORi), which target ATP-binding pocket of MTOR, and third-generation MTOR inhibitors (RapaLink-1), which combine allosteric and active site inhibition (Benjamin et al. 2011, Roux & Topisirovic 2012, Rodrik-Outmezguine et al. 2016) (Fig. 1). The new generation of MTOR inhibitors efficiently suppress EIF4EBP phosphorylation and reduce AKT signaling via inhibition of MTORC2 (Benjamin et al. 2011). These inhibitors are presently under investigation in clinical trials and are expected to exhibit enhanced efficacy in the clinic as compared to rapalogs.

In addition to MTOR inhibitors, the vast majority of oncogenic kinase inhibitors indirectly suppress MTORC1 and are thus positioned to alter the cross-talk between translational machinery and energy metabolism in neoplasia. Lapatinib is a dual receptor tyrosine kinase inhibitor (TKI) (Fig. 1) of epidermal growth factor receptor (EGFR) and ERB-B2 receptor Tyrosine kinase (EEEB2) human epidermal growth factor receptor 2 (HER2), which is used for treating HER2-positive breast cancer (Geyer et al. 2006). Lapatinib inhibits the RAS-RAF-MEK-MAPK and MTORC1 signaling pathways (Brady et al. 2015). Furthermore, lapatinib inhibits glycolysis and reduces mitochondrial membrane potential (Paech et al. 2017). Vemurafenib is a BRAF V600E serine/threonine kinase inhibitor used in the treatment of advanced melanoma (Young et al. 2012). It downregulates the RAS-RAF-MEK-MAPK and MTORC1 signaling pathways (Zhan et al. 2015) (Fig. 1). Similar to lapatinib, vemurafenib inhibits glycolysis in melanoma cells (Delgado-Goni et al. 2016). Furthermore, vemurafenib increases oxidative and anaplerotic pyruvate carboxylase (PC) mitochondrial metabolism and decreases lipid synthesis (Delgado-Goni et al. 2016). Imatinib is another TKI that suppresses abnormal activation of the PI3K/AKT/MTORC1 pathway downstream of a constitutively active BCR/ABL kinase present in chronic myelogenous leukemia (Hirao et al. 2018). Consistent with most kinase inhibitors (KI), imatinib also inhibits glucose uptake and glycolysis (Boren et al. 2001, Gottschalk et al. 2004). Moreover, its ability to alter metabolic enzyme activities involved in fatty acid and de novo nucleic acid synthesis demonstrates the mechanism by which it inhibits leukemia cell growth (Boren et al. 2001).

While KI suppress MTOR signaling, their inability to impede EIF4F complex assembly dramatically reduces their anti-neoplastic efficacy. For example, in B RAF(V600)-mutated melanoma, resistance to anti-BRAF and anti-MEK therapies, can be overturned by altering EIF4F complex activity by using EIF4A inhibitors (Boussemart et al. 2014). In a model of mammary epithelial cells, resistance to PI3K/MTOR inhibitor BEZ235 was induced by either MYC or EIF4E amplification (Ilic et al. 2011). Resistant cells showed elevated 5’ cap-dependent mRNA translation, supporting the importance of EIF4F activity in development of resistance to KI (Ilic et al. 2011). In breast cancer xenografts, overexpressing EIF4E induces resistance to ERBB2 and EGFR inhibitors including lapatinib (Zindy et al. 2011). Similarly, high EIF4F and cap-dependent translation levels occur in non-small cell lung cancer cells resistant to EGFR inhibitor erlotinib (Patel et al. 2013). More generally, a high EIF4E/EIF4EBP ratio was shown to dramatically decrease the efficacy of MTOR inhibitors across multiple cancer cell lines and in vivo (Alain et al. 2012a,b). Overall, these findings underscore the role of translation machinery in determining the efficacy of MTOR targeted therapies and suggest that the inability of such approaches to suppress mRNA translation may facilitate metabolic adaptations of cancer cells to KIs.
Combinations of KI that impinge on EIF4F (e.g. MTOR inhibitors) with oncogenic KI (e.g. TKIs) have been explored. The BOLERO-3 clinical trial has tested a combination of everolimus, a rapalog, and trastuzumab, which targets the ERBB2 receptor (Andre et al. 2014). Based on the initial results of the BOLERO-3 trial, it appears that such combination represents a promising therapeutic strategy to target patients with advanced ERBB2+ breast cancer developing resistance to conventional therapy (Andre et al. 2014). However, in a subset of patients, high EIF4E(EIF4EBP ratio may result in resistance to trastuzumab/everolimus combinations (Alain et al. 2012b). Notably, alternative possibilities of targeting the translation machinery have been developed (e.g. EIF4A inhibitors). These therapies target directly the formation of the EIF4F complex and could provide good candidates for combination with KI to manage resistance. They are discussed in more detail in the next section.

Cancer metabolism and therapeutic implications

Although metabolic reprogramming in cancer is thought to provide sufficient therapeutic window to selectively target malignant cells, while not causing excessive toxicity in normal tissues, changes in metabolic and associated translational programs are also linked to the development of drug resistance (Zhao et al. 2010, Han et al. 2015, Deblois et al. 2016). For example, sustained MTOR activation observed in SKBr3 lapatinib-resistant cells, leads to dysregulated expression of ESSRA, which mediates lapatinib resistance through increased glutamine metabolism and ROS detoxification (Deblois et al. 2016). Moreover, ESSRA mediates the intrinsic resistance of breast cancer cells to PI3K/MTOR inhibitors (Park et al. 2016). ESSR 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 (Poulikakos et al. 2011, Boussmart et al. 2014). 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 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 KI, which is further exacerbated by the seemingly outstanding plasticity of malignant metabolomes.

Strategies to overcome metabolic adaptations of cancer cells to KI, whereby combinatory drugs are used to disrupt metabolic reprogramming processes which underpin development of resistance, are being developed. For example, the inhibition of ESSRA 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 (Fig. 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 (Javesghani et al. 2012). Hence, there is a rationale for combining BRAF inhibitors (BRAFi), which suppress glycolysis, and biguanides (Zhao et al. 2010). Indeed, phenformin – a more potent inhibitor of mitochondrial complex I – and BRAF inhibitors exhibits synergistic anti-tumorigenic effects in melanoma (Yuan et al. 2013, Bridges et al. 2014). Furthermore, BRAFi resistant melanoma cells have an increased reliance on glutaminolysis, as they were shown to be more sensitive to glutamine starvation and glutaminase inhibitors compared to BRAFi-sensitive cells (Hernandez-Davies et al. 2015, Baenke et al. 2016). These examples suggest that using drug combinations that alter metabolic adaptations which underlie resistance to KIs may constitute an effective therapeutic strategy.

Consistently, combination of phenformin with various KI (lapatinib, vemurafenib, imatinib) results in synergistic anti-proliferative effects, which are paralleled by MTORC1 inhibition, disruption of the EIF4F complex and the downregulation of the translational control of genes involved in non-essential amino acid synthesis (NEAA) (serine, aspartate, asparagine): phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), PC and ASNS (Hulea et al., Cell Metabolism, in press; bioRxiv 160879; doi: https://doi.org/10.1101/160879) (Fig. 2). However, cells lacking EIF4EBP1 and EIF4EBP2, in which MTORC1 inhibition is uncoupled from EIF4F disassembly, show dramatically reduced sensitivity to the phenformin/KI combinations.
enzymes (Hulea et al., Cell Metabolism, in press; bioRxiv 160879; doi: https://doi.org/10.1101/160879). This at least in part is a consequence of their inability to suppress NEAA biosynthesis and translation and transcription of mRNAs encoding corresponding enzymes (Hulea et al., Cell Metabolism, in press; bioRxiv 160879; doi: https://doi.org/10.1101/160879). A similar phenomenon was observed in cells depleted of von Hippel–Lindau (VHL) tumor suppressor, the major regulator of HIF1A protein stability (Semenza 2007). VHL-null cells maintain high HIF1A protein levels under normoxic conditions (Maxwell et al. 1999) and are less sensitive to the phenformin/KI combinations, at least in part due to changes in the glutamine metabolic program in these cells (Hulea et al., Cell Metabolism, in press; bioRxiv 160879; doi: https://doi.org/10.1101/160879). Collectively, these findings emphasize the plasticity of translational and metabolic programs of cancer cells, which allows them to rapidly adapt to therapeutic insults.

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

In the context of combination therapy with drugs inducing energetic stress, therapies resulting in reduced energy consumption, leading to metabolic dormancy and a cytostatic effect, are not very effective. Therefore, in order to effectively kill cancer cells by inducing energetic 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).

**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 signaling 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**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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