mTOR: Target of Opportunity?

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White Paper No 170
January 2020

mTOR is a protein that sits in the midst of a multiplicity of paths and it is acted upon in a variety of ways and it then acts upon others in a similar variety. We examine the current status of mTOR and its application to a variety of Cancers.

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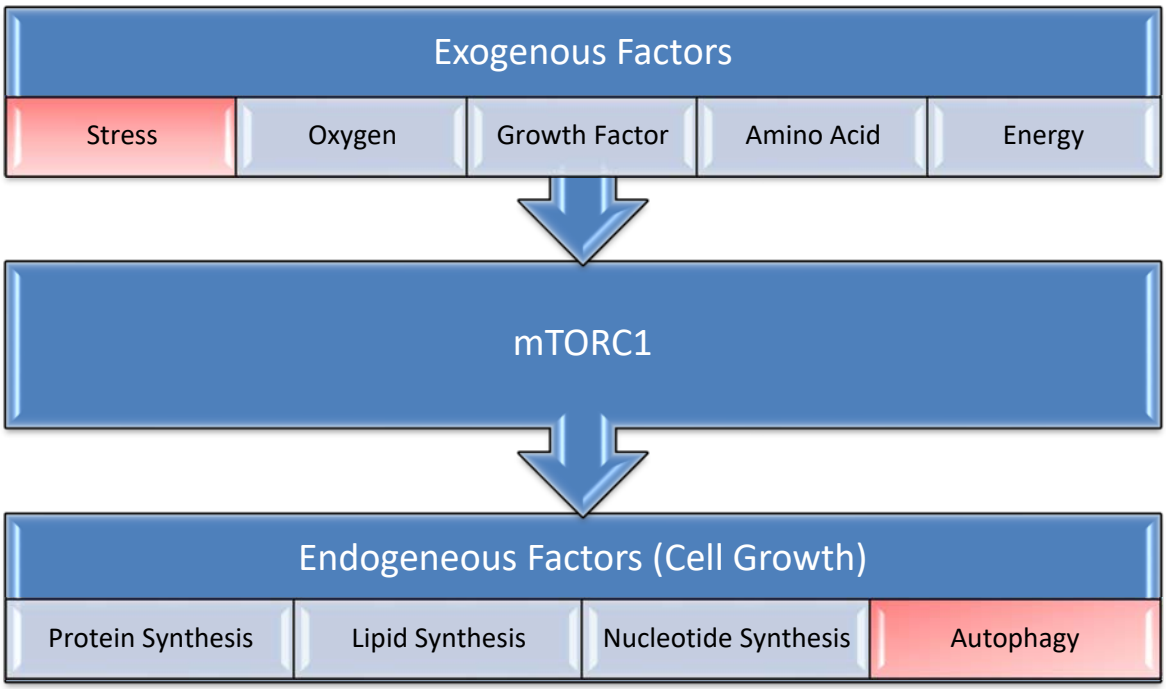
Contents

1 INTRODUCTION

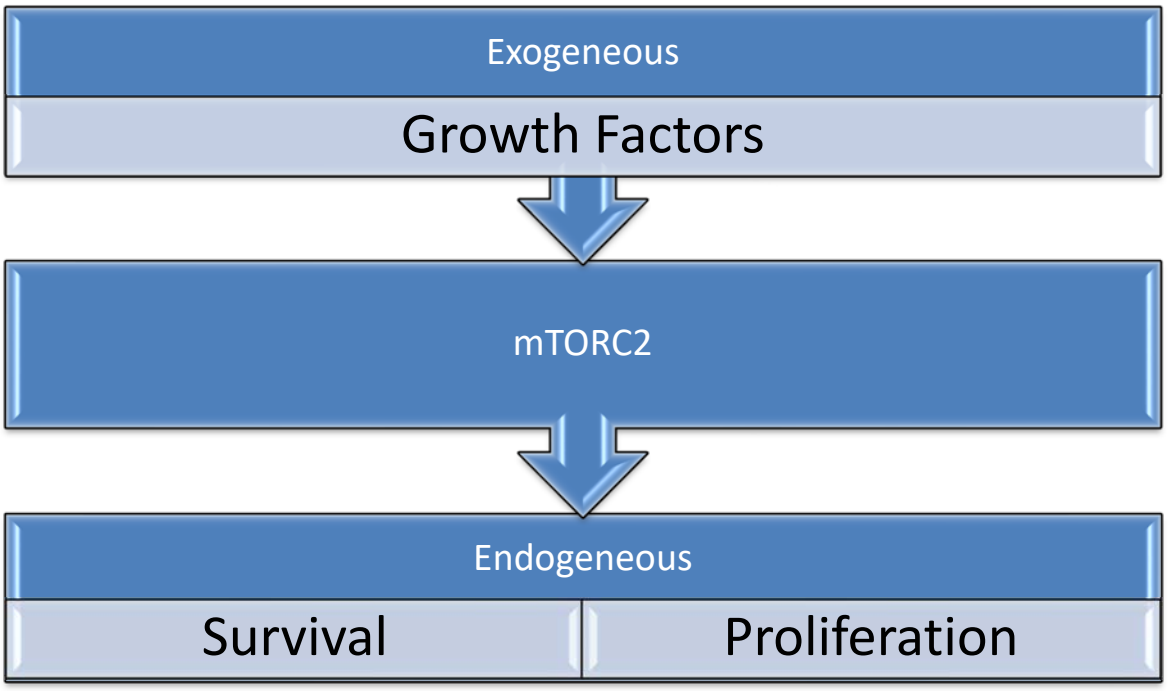
There are a multiplicity of targets which have been explored over the past twenty years or so for the treatment of cancers. mTOR is one of them and there are a wealth of reasons for its interest. In this Note we explore mTOR in some detail and do so as a continuation of targets.

Historically, mTOR, the mammalian target of rapamycin or now called the mechanical target of rapamycin, was found after the finding of rapamycin, a fungal derivative found on Easter Island. Rapamycin targets mTOR and it was the result of the effects of this targeting that mTOR itself was identified. Yet it is not mTOR per se that does the work but a heterodimer of mTOR, actually two, mTORC1 and mTORC2. It is this concatenation of products that we examine.

From Saxton and Sabatini, we have the following exogeneous and endogenous factors related to each of these mTOR complexes. Note that mTORC1 has the most influence. The inputs or exogenous factors are what drive the effects of mTOR elements.



In a similar manner we have the following for mTORC2.



The paradigms above place mTOR and its complexes in a central role of controlling cell proliferation, growth and survival. The key question is; is mTOR and its complexes a major player in the development of proliferation or is it merely a participant driven by other elements? Is the identification of mTOR and its complexes perhaps an over-emphasized target or a critical one worthy of control and suppression?

The overall objectives of this Note are as follows:

1. Review the mTOR constructs

2. Discuss rapamycin and its uses

3. Examine the mTOR related pathways to understand the input and output factors

4. Examine the various cancers putatively impacted on by mTOR

5. Examine the various therapeutics related to mTOR targeting

6. Consider the interactions between mTOR and a multiplicity of miRNAs

However, the key observation is that mTOR and its complexes act as a result to some driver action and act upon downstream entities. It is not mTOR that changes or mutates and does something different. It is mTOR and its complexes doing what they are supposed to do. Thus this discussion is not about some mutation or similar change in a gene and its product. It is about a gene product doing what it should but driven or driving other entities resulting in a malignant performance.

2 MTOR

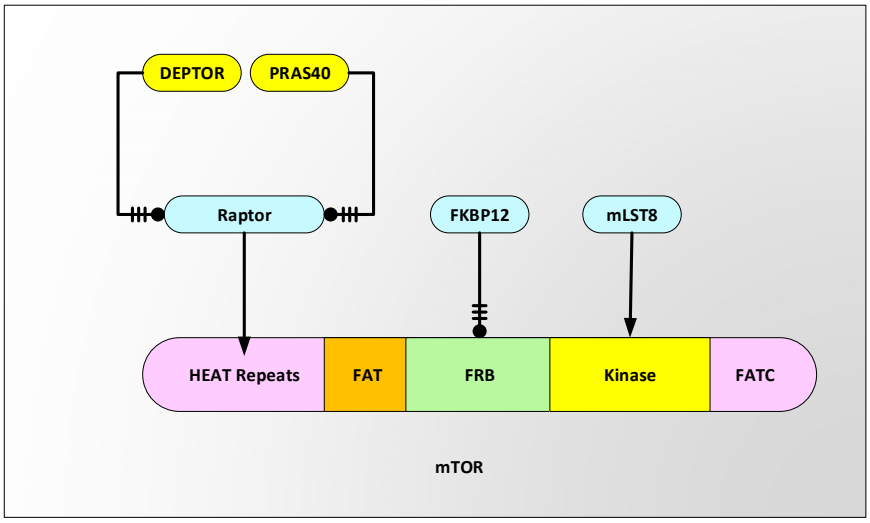
We start with a brief overview of mTOR. As NCBI states[1]:

The protein encoded by this gene belongs to a family of phosphatidylinositol kinase-related kinases. These kinases mediate cellular responses to stresses such as DNA damage and nutrient deprivation. This protein acts as the target for the cell-cycle arrest and immunosuppressive effects of the FKBP12-rapamycin complex.

Now mTOR by itself plays a role only when conjugated with other products, namely those generating mTORC1 and mTORC2. We now briefly explain the structure of each of these two.

2.1 mTORC1

As we noted earlier mTORC1 has the most significant set of impacts on cell stability. Also as we noted there are upstream and downstream influences generated by this complex. We start with the structure of the mTORC1 complex as noted below:



The mTOR protein is composed of five sections, including the kinase element. The HEAT Repeats, as noted by Neuwald and Hirano are:

HEAT repeats correspond to tandemly arranged curlicue-like structures that appear to serve as flexible scaffolding on which other components can assemble. Using sensitive sequence analysis techniques we detected HEAT repeats in various chromosome-associated proteins, including four families of proteins associated with condensins and cohesins, which are nuclear complexes that contain structural maintenance of chromosome (SMC) proteins.

[1] https://www.ncbi.nlm.nih.gov/gene/2475

RAPTOR is the regulatory associated protein of mTOR[2]. RAPTOR is an mTOR binding protein.

As Saxton and Sabatini have noted:

In order to grow and divide, cells must increase production of proteins, lipids, and nucleotides while also suppressing catabolic pathways such as autophagy. mTORC1 plays a central role in regulating all of these processes and therefore controls the balance between anabolism and catabolism in response to environmental conditions... the critical substrates and cellular processes downstream of mTORC1 and how they contribute to cell growth.

Most of the functions discussed here were identified and characterized in the context of mammalian cell lines, while the physiological context in which these processes are important will be discussed in greater detail below.

Protein Synthesis mTORC1 promotes protein synthesis largely through the phosphorylation of two key effectors, p70S6 Kinase 1 (S6K1) and eIF4E Binding Protein (4EBP). mTORC1 directly phosphorylates S6K1 on its hydrophobic motif site, Thr389, enabling its subsequent phosphorylation and activation by PDK1.

S6K1 phosphorylates and activates several substrates that promote mRNA translation initiation, including eIF4B, a positive regulator of the 50cap binding eIF4F complex. S6K1 also phosphorylates and promotes the degradation of PDCD4, an inhibitor of eIF4B, and enhances the translation efficiency of spliced mRNAs via its interaction with SKAR, a component of exon-junction complexes.

The mTORC1 substrate 4EBP is unrelated to S6K1 and inhibits translation by binding and sequestering eIF4E to prevent assembly of the eIF4F complex. mTORC1 phosphorylates 4EBP at multiple sites to trigger its dissociation from eIF4E, allowing 50cap-dependent mRNA translation to occur.

Although it has long been appreciated that mTORC1 signaling regulates mRNA translation, whether and how it affects specific classes of mRNA transcripts has been debated. Global ribosome footprinting analyses, however, revealed that, while acute mTOR inhibition moderately suppresses general mRNA translation, it most profoundly affects mRNAs containing pyrimidine-rich 50 TOP or ''TOP-like'' motifs, which includes most genes involved in protein synthesis

Now the upstream influencers or drivers are detailed below from Seeboeck et al:

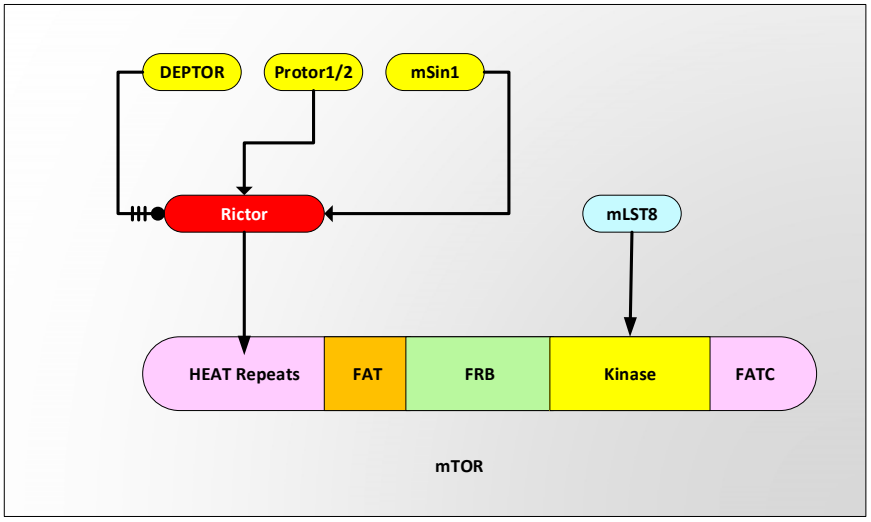
[2] https://www.ncbi.nlm.nih.gov/gene/57521

mTORC1 Upstream	
Rapamycin	rapamycin
FKBP12	FK506-binding protein 12 kDa
TSC	tuberous sclerosis complex
Rheb	Ras homolog enriched in brain
IGF-1 pathway	insulin/insulin like growth factor
AKT	AKT serine/threonine kinase
mTORC2	promotes dissociation of PRAS40 from mTORC1.
Wnt	Wnt
TNFα 1	tumor necrosis factor α
AMPK	5'-AMP-activated protein kinase
REDD1	regulated in development and DNA damage responses 1

The above each in their own manner effects the actions of mTORC1. Rapamycin is a major driver when present. Some of these are exogenous to the cell itself such as the growth factors and others are part of the cell normal pathway. Note that mTORC2 has a driving factor as well. We shall briefly explore that next.

2.2 mTORC2

Now we consider mTORC2. From Seeboeck et al the structure appears as below:



Rictor is akin to Raptor. We see the same underlying mTOR base elements and then the complex binding to create the multiprotein complex. Now the drivers or upstream elements are shown below. Like mTORC1, it also is a driver here.

mTORC2 Upstream	
Rapamycin	rapamycin
FKBP12	FK506-binding protein 12 kDa
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
AKT	AKT serine/threonine kinase
mTORC1	Negative feedback loop between mTORC1 and insulin/PI3K signaling

Saxton and Sabatini have noted the downstream effects of mTORC2:

While mTORC1 regulates cell growth and metabolism, mTORC2 instead controls proliferation and survival primarily by phosphorylating several members of the AGC (PKA/PKG/PKC) family of protein kinases.

The first mTORC2 substrate to be identified was PKCa, a regulator of the actin cytoskeleton. More recently, mTORC2 has also been shown to phosphorylate several other members of the PKC family, including PKCd, PKCz, as well as PKCg and PKCε, all of which regulate various aspects of cytoskeletal remodeling and cell migration.

The most important role of mTORC2, however, is likely the phosphorylation and activation of Akt, a key effector of insulin/ PI3K signaling.

Once active, Akt promotes cell survival, proliferation, and growth through the phosphorylation and inhibition of several key substrates, including the FoxO1/3a transcription factors, the metabolic regulator GSK3b, and the mTORC1 inhibitor TSC2.

However, while mTORC2- dependent phosphorylation is required for Akt to phosphorylate some substrates in vivo, such as FoxO1/3a, it is dispensable for the phosphorylation of others, including TSC2. Finally, mTORC2 also phosphorylates and activates SGK1, another AGC-kinase that regulates ion transport as well as cell survival.

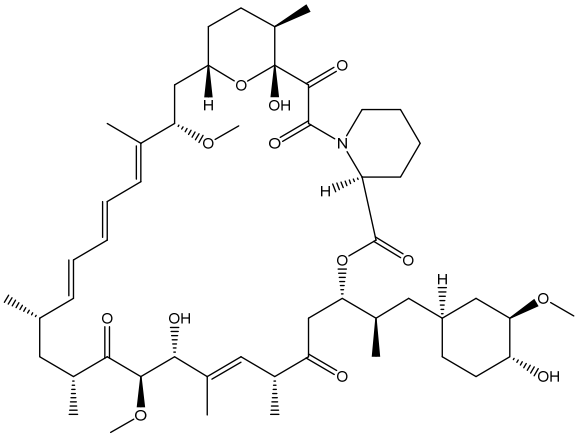
The mTORC1-dependent shift toward increased anabolism should only occur in the presence of pro-growth endocrine signals as well as sufficient energy and chemical building blocks for macromolecular synthesis. In mammals, these inputs are largely dependent on diet, such that mTORC1 is activated following feeding to promote growth and energy storage in tissues such as the liver and muscle but inhibited during fasting conserve limited resources. Here, we discuss the cellular pathways upstream of mTORC1 and the mechanisms through which they control mTORC1 activation.

3 RAPAMYCIN

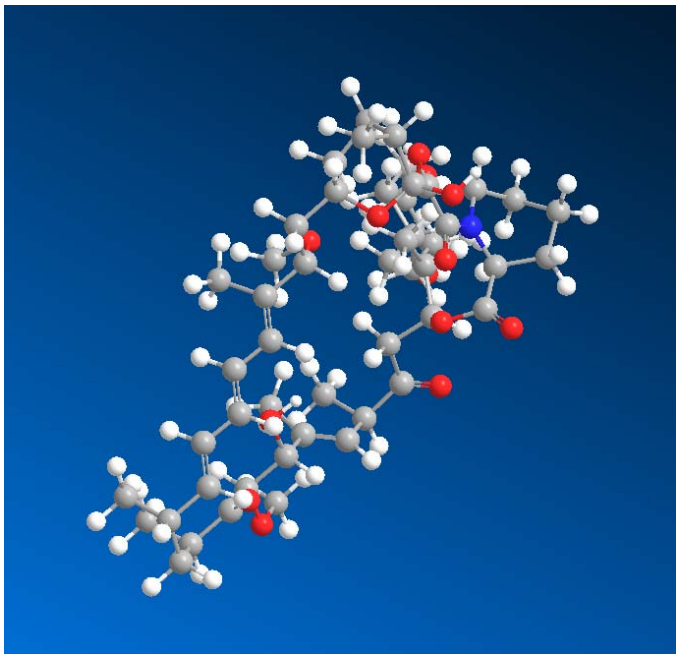
As we noted earlier rapamycin is a fungal derivative which has aggressive properties in blocking mTOR and thus its effects on cellular behavior. Various therapeutic derived from rapamycin, rapalogs, have been developed and used clinically. We briefly summarize rapamycin.

3.1 STRUCTURE

The Rapamycin formula appears below. This is the structure of the naturally occurring molecule.



The three dimensional structure of Rapamycin follows below:



There are well identified binding areas and these then are what we see as the major pathway upstream control factors.

3.2 ACTIONS

Understanding rapamycin and its adjuncts is essential to understanding the actions of mTOR. We present a few summaries herein. As Li et al noted:

Increased activation of mTORC1 is observed in numerous human cancers due to gain-offunction mutations in oncogenes (i.e., PI3K, AKT, or Ras) and/or loss-of-function mutations in tumor suppressors (i.e., PTEN, LKB1 or TSC1/2), upstream regulators of mTORC1. These mutations provide cancer cells with a selective growth advantage in comparison to normal cells (Menon and Manning, 2008). In order to meet the high demands of proliferation, cancer cells often have fundamental alterations in nutrient uptake and energy metabolism, processes that are directly controlled by the mTORC1 pathway.

Accordingly, in addition to driving protein synthesis, oncogenic activation of mTORC1 promotes a gene expression program that is involved in cancer cell metabolic reprogramming. Activation of mTORC1 promotes glycolysis via upregulation of Hypoxia-inducible factor alpha (HIF1α) and c-Myc; stimulates lipid biosynthesis and the pentose phosphate pathway through sterol regulatory element binding protein 1 (SREBP-1); and positively controls glutamine metabolism by SIRT4 repression.

Thus, drugs that selectively target mTORC1, like rapamycin, are expected to impair cancer metabolism and are considered promising anti-cancer therapies. The poor solubility and pharmacokinetics of rapamycin triggered the development of several rapamycin analogs (rapalogs).

Two water-soluble derivatives of rapamycin, temsirolimus and everolimus, were approved by the Food and Drug Administration (FDA) in 2007 and 2009, respectively, for the treatment of advanced renal cancer carcinoma (RCC). In 2011, the FDA approved the use of everolimus for patients with progressive neuroendocrine tumors of pancreatic origin (PNET). Additionally, temsirolimus was evaluated in several clinical trials for the treatment of advanced neuroendocrine carcinoma (NEC), advanced or recurrent endometrial cancer, and relapsed or refractory mantle cell lymphoma.

Moreover, a few trials of everolimus were conducted in patients with advanced gastric cancer, advanced non-small cell lung cancer (NSCLC), and advanced hepatocellular carcinoma. Ridaforolimus, a rapamycin analog, was also examined in clinical trials for advanced bone and soft-tissue sarcomas as well as a variety of advanced solid tumors.

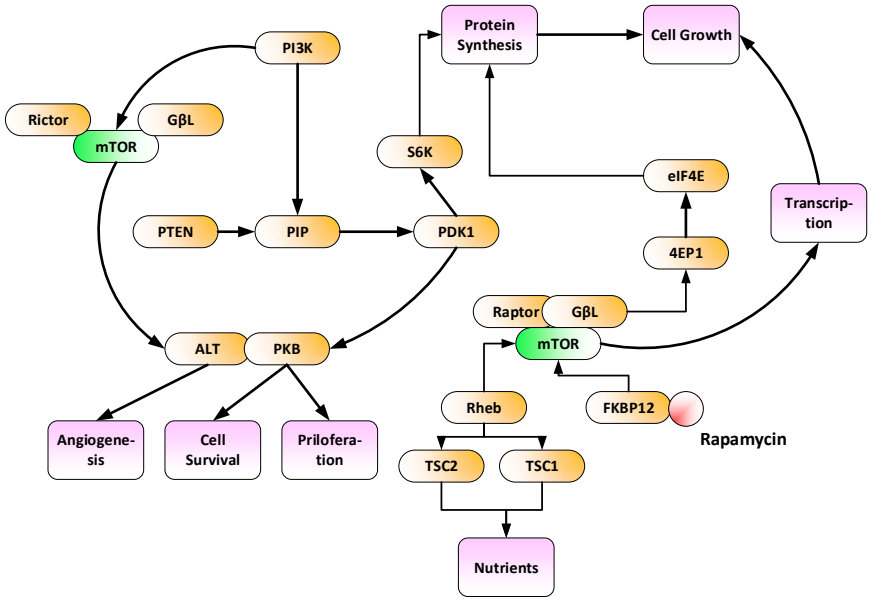
Overall, however, ***rapalogs have only achieved modest effects in major solid tumors in the clinic. The reasons for the limited clinical success of rapalogs have not been established, but are likely related to the large number of mTORC1-regulated negative feedback loops that suppress upstream signaling systems such as activation of receptor tyrosine kinases****, PI3K-Akt signaling and Ras-ERK pathway and which can be re-activated with rapamycin (discussed more extensively below). In order to overcome these limitations, alternative strategies have been explored in the past few years.*

The feedback loops are partially displayed herein. The mTOR complexes are indeed in complex loops and one suspects they may be even more complex than we have currently displayed them.

For instance, a number of ATP-competitive mTOR inhibitors have been developed, blocking both mTORC1 and mTORC2 activity. *Due to high sequence homology shared between mTOR and PI3K, some compounds that were originally identified as PI3K inhibitors were later shown to inhibit mTOR. Unlike rapamycin, which is a specific allosteric inhibitor of mTORC1, these ATP-competitive inhibitors target the catalytic site and prevent the feedback-mediated PI3K/Akt activation (described below), and therefore can potentially offer broader, more potent and sustained mTOR inhibition.*

4 mTOR PATHWAYS

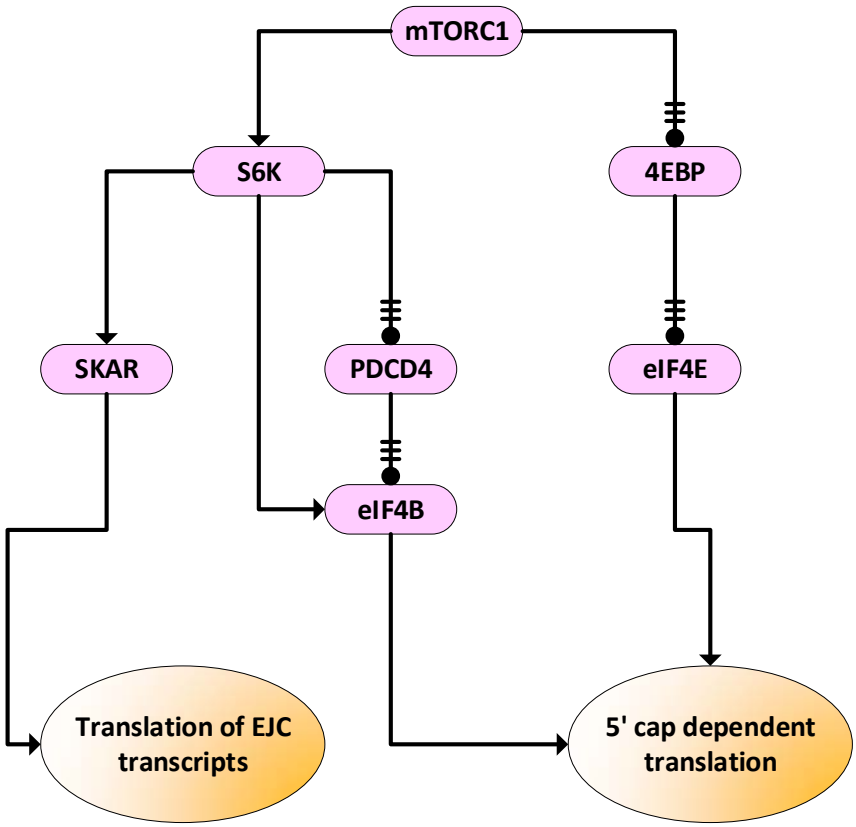
We now consider the mTOR pathways. These are the effecting paths, namely those for which mTOR has influence on cell behavior. We show mTOR with separate Raptor and Rictor conjugates. As noted above they are the fundamental elements of the mTORC1 and mTORC2 conjugates. We shall examine these pathways for several malignancies.



We have briefly stated the drivers of the mTOR complexes and their resultant impact processes. The specific details are generally understood but like many of these processes there are specifics which are yet to be detailed. We now provide some of the specifics as currently understood for three major functions.

4.1 RNA TRANSLATION

Let us first consider the impact on RNA translation. The figure below demonstrates several of the steps in this process.



Saxton and Sabatini remark on the above as follows:

mTORC1 promotes protein synthesis largely through the phosphorylation of two key effectors, p70S6 Kinase 1 (S6K1) and eIF4E Binding Protein (4EBP. mTORC1 directly phosphorylates S6K1 on its hydrophobic motif site, Thr389, enabling its subsequent phosphorylation and activation by PDK1. S6K1 phosphorylates and activates several substrates that promote mRNA translation initiation, including eIF4B, a positive regulator of the 50cap binding eIF4F complex.

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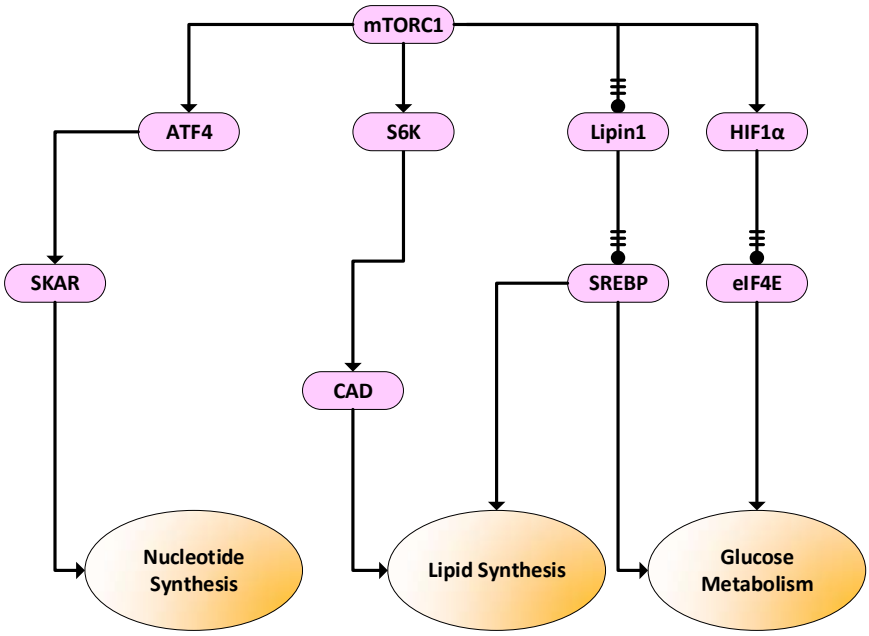
Although it has long been appreciated that mTORC1 signaling regulates mRNA translation, whether and how it affects specific classes of mRNA transcripts has been debated. Global ribosome footprinting analyses, however, revealed that, while acute mTOR inhibition moderately suppresses general mRNA translation, it most profoundly affects mRNAs containing pyrimidine-rich 50 TOP or ''TOP-like'' motifs, which includes most genes involved in protein synthesis

The above complex network is significant in that mTORC1 has substantial but intricate controls over the RNA translation.

4.2 METABOLISM

Finally we can show how mTORC1 also plays a significant role in cellular metabolism. These are shown below.



Again, as Saxton and Sabatini note:

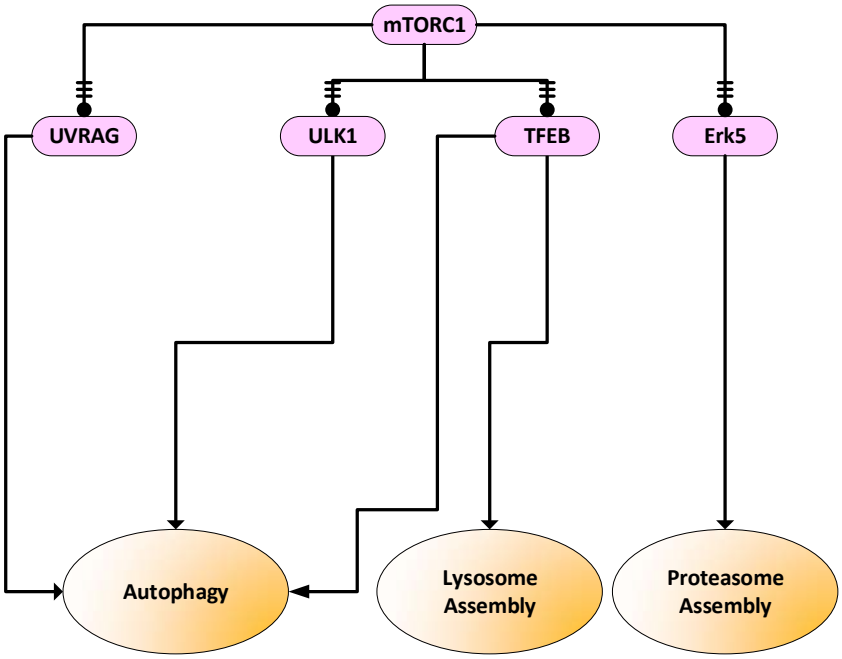
Recent studies established that mTORC1 also promotes the synthesis of nucleotides required for DNA replication and ribosome biogenesis in growing and proliferating cells. mTORC1 increases the ATF4-dependent expression of MTHFD2, a key component of the mitochondrial tetrahydrofolate cycle that provides one carbon units for purine synthesis.

Additionally, S6K1 phosphorylates and activates carbamoyl-phosphate synthetase (CAD), a critical component of the de novo pyrimidine synthesis pathway. mTORC1 also facilitates growth by promoting a shift in glucose metabolism from oxidative phosphorylation to glycolysis, which likely facilitates the incorporation of nutrients into new biomass. mTORC1 increases the translation of the transcription factor HIF1a which drives the expression of several glycolytic enzymes such as phospho-fructo kinase (PFK).

Furthermore, mTORC1- dependent activation of SREBP leads to increased flux through the oxidative pentose phosphate pathway (PPP), which utilizes carbons from glucose to generate NADPH and other intermediary metabolites needed for proliferation and growth.

4.3 PROTEIN CONTROL

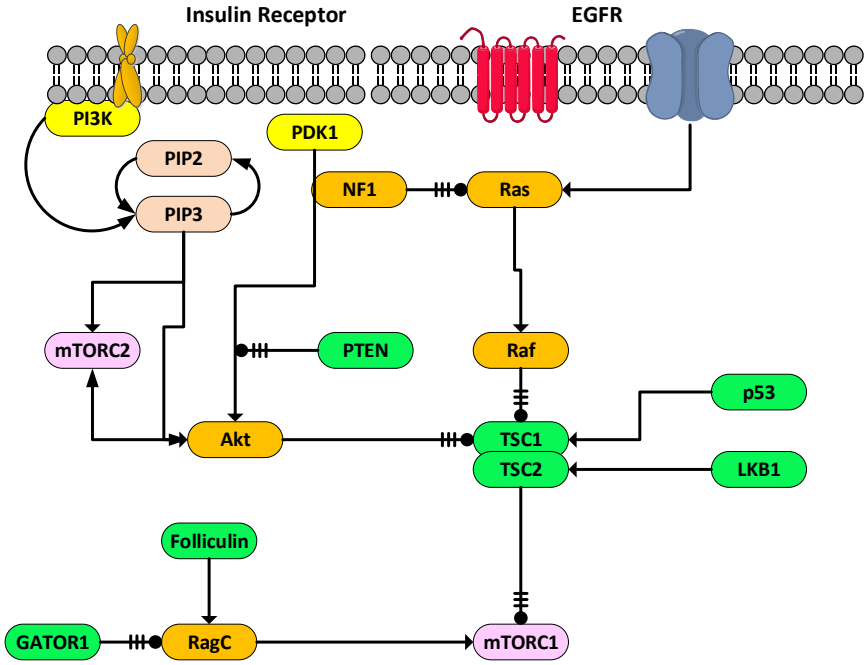
A third major control mechanism is that of proteins. We demonstrate this below:



Autophagy is necessary for homeostasis and loss of that control can be a key element in metastatic growth. Lysosome and proteasome assembly are also key elements in cell progression.

5 mTOR CONTROLLED CANCERS

As can be seen from the above, mTOR and its complexes play a variety of roles in cell homeostasis. Any significant disruption of those processes may lead to the development of a variety of cancers. The major mTOR complex pathways are shown below:



We know from the study of many cancers that products like PTEN, RAF/BRAF, p53 and others can control malignant behavior. However we also know from above that activation of the mTOR conjugates have significant downstream effects. Thus one of the issues we are concerned with is what drives the process? As Saxton and Sabatini have noted:

As discussed above, mTORC1 functions as a downstream effector for many frequently mutated oncogenic pathways, including the PI3K/Akt pathway as well as the Ras/Raf/Mek/ Erk (MAPK) pathway, resulting in mTORC1 hyperactivation in a high percentage of human cancers.

Namely mTOR per se does not have to be mutated but that the genes preceding it upstream when mutated can make mTOR over activated.

Furthermore, the common tumor suppressors TP53 and LKB1 are negative regulators of mTORC1 upstream of TSC1[3] *and TSC2*[4]*, which are also tumor suppressors originally identified through genetic analysis of the familial cancer syndrome TSC. Several components of the nutrient sensing input to mTORC1 have also been implicated in cancer progression, including all three subunits of the GATOR1 complex, which are mutated with low frequency in glioblastoma, as well as RagC, which was recently found to be mutated at high frequency (18%) in follicular lymphoma.*

Additionally, mutations in the gene encoding folliculin (FLCN) are the causative lesion in the Birt-Hogg-Dube hereditary cancer syndrome, which manifests similarly to TSC. Finally, mutations in MTOR itself are also found in a variety of cancer subtypes, consistent with a role for mTOR in tumorigenesis.

mTORC2 signaling is also implicated in cancer largely due to its role in activating Akt, which drives pro-proliferative processes such as glucose uptake and glycolysis while also inhibiting apoptosis. Indeed, at least some PI3K/Akt-driven tumors appear to rely on mTORC2 activity, as Rictor is essential in mouse models of prostate cancer driven by PTEN loss, as well as in human prostate cancer cell lines that lack PTEN.

The above is the most significant observation. The focus on mTORC1 often overshadows the major effects of mTORC2. Also there is the mutual interaction between the two.

The above observation is a critical one. It appears that upstream activation and mTOR responses are critical. We have not shown in the above the mTORC2 activation of Akt. Indeed if such be the case the impact would be significant.

While many mTORC1-driven processes likely contribute to tumorigenesis, the translational program initiated by the phosphorylation of 4EBP is likely the most critical, *at least in mouse models of Akt-driven prostate cancer and T cell lymphoma. Consistent with this, a variety of Akt and Erk-driven cancer cell lines are dependent on 4EBP phosphorylation, and the ratio of 4EBP to eIF4E expression correlates well with their sensitivity to mTOR inhibitors. The first mTOR inhibitors approved for use in cancer were a class of rapamycin derivatives known as ‘‘rapalogs.’’*

Again, there are other suppositions regarding the mTOR complexes.

[3] See NCBI https://www.ncbi.nlm.nih.gov/gene/7248 *This gene is a tumor suppressor gene that encodes the growth inhibitory protein hamartin. The encoded protein interacts with and stabilizes the GTPase activating protein tuberin. This hamartin-tuberin complex negatively regulates mammalian target of rapamycin complex 1 (mTORC1) signalling which is a major regulator of anabolic cell growth. This protein also functions as a co-chaperone for Hsp90 that inhibits its ATPase activity. This protein functions as a facilitator of Hsp90-mediated folding of kinase and non-kinase clients, including Tsc2 and thereby preventing their ubiquitination and proteasomal degradation.*

[4] See NCBI https://www.ncbi.nlm.nih.gov/gene/7249 *Mutations in this gene lead to tuberous sclerosis complex. Its gene product is believed to be a tumor suppressor and is able to stimulate specific GTPases. The protein associates with hamartin in a cytosolic complex, possibly acting as a chaperone for hamartin. Alternative splicing results in multiple transcript variants encoding different isoforms.*

The rapalog temsirolimus was first approved for treatment of advanced renal cell carcinoma in 2007, followed by everolimus in 2009. Although a small number of ''extraordinary responders'' have been reported, these rapalogs have been less successful in the clinic than anticipated from pre-clinical cancer models.

From Seeboeck et al:

Considering how involved the mTOR signaling pathway is, it comes as no surprise that it also plays a crucial role in human disease, particularly cancer. The complex most commonly associated with cell proliferation and cancer progression when deregulated is the mTORC1 complex. A number of signaling components both upstream and downstream of mTOR are frequently deregulated or altered in human cancer. Through alterations in one or multiple of these elements, mTOR signaling is activated in many cancer types, suggesting mTOR as a potent target for cancer therapy.

Due to this fact, mTOR pathway inhibitors have been of prime interest in recent years. These inhibitors include rapamycin and its analogs (rapalogs) and, more recently, mTOR kinase domain inhibitors. Despite showing promise, rapalog monotherapy has been proven mostly insufficient in causing tumor regression, with notable exceptions of tumors showing mutations in mTOR itself, LOF mutations in TSC1 or TSC2.

… reports correlating mTOR pathway mutations to drug response are yet missing, but there are studies towards that aim that are very promising. Specifically, a study identified 33 MTOR mutations that lead to pathway hyperactivity in cancer. A heightened rapamycin sensitivity in cells harboring these hyperactivating mTOR mutations suggests that they convey mTOR pathway dependency.

These results are supported by the report of an extraordinary responder with two activating mTOR mutations in urothelial carcinoma and an exceptional response to rapalog treatment in combination with a TKI. Furthermore, patients with the genetic disorder tuberous sclerosis complex (TSC) (mutations in the TSC1 or TSC2 gene), commonly develop tumors like astrocytomas or angiomyolipomas as well as the related lung disorder Lymphangioleiomyomatosis (LAM).

Treatment with rapalogs has been shown to improve clinical outcomes and cause tumor regression in TSC patients with astrocytomas or sporadic LAM, again suggesting a dependence on mTOR signaling for tumor growth. A phase II clinical trial found a 50% response rate in TSC patients with angiomyolipomas or sporadic LAM.

Furthermore, heightened treatment sensitivity was associated with TSC1 or TSC2 LOF mutations, as reported in bladder and thyroid cancer.

Other responders have been reported in one pancreatic cancer with loss of suppression of mTOR signaling and three patients with perivascular epithelioid cell tumors with the loss of TSC2.

However, in the thyroid cancer extraordinary responder case study, the tumor gained resistance to rapalog treatment as it acquired a mutation in mTOR, which prevented the binding of the

rapalog, as well as a nonsense mutation in TSC2. Further literature regarding rapamycin and rapalogs as monotherapy….

These specific cases show the importance of rapamycin and rapalogs, as well as the development of reliable biomarkers, for precision medicine. Apart from these cases, it has been shown that, while not very potent on its own, mTORC1 inhibition might be necessary to achieve a proper response to drugs that target the primary oncogenic pathway in the given cancer. On top of that, sustained mTORC1 activation is proposed to be a major mechanism of resistance to targeted therapies.

The same authors have a Table depicting related genes and specific cancers (as modified):

Gene	***Site(s)***
AKT1	breast, skin, urinary tract, colon, lung
eIF4g	colon, lung
mTOR	colon, endometrium, skin, kidney
NF1	skin, soft tissue urinary tract, lung, colon
PIK3CA	breast, endometrium, urinary tract, colon
PIK3CG	skin, colon, lung
PIK3R1	breast, endometrium prostate, leukemia
PKC beta	lung, skin, colon
PTEN	breast, endometrium prostate, leukemia
Raptor	various
Rictor	lung, breast
TP53	solid cancer, leukemia lymphoma, melanoma
TSC1	skin, urinary tract, liver
TSC2	liver, breast
VHL	kidney, neuroendocrine tumors

As Hua et al note:

The activity of mTOR is frequently upregulated in human cancer. The aberrant activation of mTOR in human cancer may be attributed to mTOR pathway-activating mutations, amplification, or overexpression of the components of mTOR complexes and mutations or loss of negative regulators of mTOR. PIK3CA mutations are frequently detected in human cancer. Activation of PI3K promotes both mTORC1 and mTORC2 activation.

In addition, mutations in KRAS and BRAF may lead to mTORC1 activation. Especially, KRAS can directly bind to PIK3CA (p110α) and activates PI3K pathway, leading to mTOR activation. mTOR-activating mutations are observed in kidney cancer.

While mTOR activity is usually upregulated by growth factors and amino acids, activating mutations in mTOR may result in RAG- and RHEB independent mTOR hyperactivation, thus loss of the dependency on growth factors and amino acid. Point mutations in RHEB and GATOR1 were also detected in renal cancer and endometrial cancer. RHEB1 is overexpressed in acute myeloid leukemia (AML) and promotes AML progression.

Whereas mTOR amplification is rare in human cancer, rictor amplification is detected in various kinds of cancer, such as breast cancer, gastric cancer, and liver cancer. Moreover, rictor is overexpressed in human cancers of the brain, breast, lung, gastric, colon, liver, and tongue. Given that mTOR has critical roles in tumor progression, mTOR inhibitors hold promise in cancer therapy. Indeed, rapamycin analogs (rapalog) have been approved for treating cancer in the clinic. In addition, many mTOR inhibitors with different mechanisms of action have been developed, some of which are undergoing clinical trials in variety types of human cancer.

Rapalog Rapamycin was originally identified as an antifungal, immunosuppressive, and antiproliferative agent. Later studies revealed that rapamycin binds to the 12 kDa FK506-binding protein (FKBP12) and then inhibits mTORC1. Since rapamycin has poor solubility and pharmacokinetics, it is not suitable for treating human cancer. So far, several water-soluble rapamycin analogs have been developed. For example, temsirolimus and everolimus exhibit tumor-suppressive effects in vivo.

Both temsirolimus and everolimus have been used to treat advanced renal cell carcinoma (RCC) in the clinic. Moreover, everolimus is prescribed for treating pancreatic neuroendocrine tumors and advanced breast cancer. Besides, there are many clinical trials to evaluate the efficacy of rapalogs in treating other types of human cancer, such as advanced gastric cancer, hepatocellular carcinoma, non-small cell lung cancer, endometrial cancer, and mantle cell lymphoma

We will return later to the various therapeutic options as noted above. We now briefly summarize mTOR impacts on a variety of cancers.

5.1 LIVER

Liver cancer, primary and not metastatic, is often the result of viral infections. As Villanueva notes:

Hepatocellular carcinoma accounts for the majority of primary liver cancers. Worldwide, liver cancers are the fourth most common cause of cancer-related death and rank sixth in terms of incident cases. On the basis of annual projections, the World Health Organization estimates that more than 1 million patients will die from liver cancer in 2030.2 In the United States, the rate of death from liver cancer increased by 43% (from 7.2 to 10.3 deaths per 100,000) between 2000 and 2016.3 With a 5-year survival of 18%, liver cancer is the second most lethal tumor, after pancreatic cancer.

The majority of hepatocellular carcinomas occur in patients with underlying liver disease, mostly as a result of hepatitis B or C virus (HBV or HCV) infection or alcohol abuse. Universal HBV vaccination and wide implementation of direct-acting antiviral agents against HCV are likely to change the etiologic landscape of hepatocellular carcinoma. However, the increase in nonalcoholic fatty liver disease (NAFLD), which together with metabolic syndrome and obesity amplifies the risk of liver cancer, will soon become a leading cause of liver cancer in Western countries.

Racial or ethnic group differences play an important role in the probability of survival, with blacks and Hispanics less likely than whites to undergo curative therapies.6 Systemic therapies for patients with an advanced stage of liver cancer are rapidly changing, with four new agents showing clinical efficacy in phase 3 trials in the past 2 years….

The molecular subtypes can be grouped in two main classes: the proliferation class and the nonproliferation class. The proliferation class, more commonly seen in patients with HBV infection, is characterized by molecular and histologic features that result in aggressive clinical behavior, including high serum levels of alphafetoprotein, poor cell differentiation, chromosomal instability, TP53 mutations, and activation of oncogenic pathways (e.g., RAS–mitogen-activated protein kinase [MAPK], AKT–mammalian target of rapamycin [mTOR], and MET [a hepatocyte growth factor receptor]). Most of the gene signatures associated with a poor clinical outcome are also enriched in the proliferation class

The comment above is a clear reflection of the supportive role of mTOR. As Li et al have noted regarding liver cancer:

Inhibition of mTORC1 and mTORC2 could attenuate migration and invasion; PtoxDpt[5]-induced migration and invasion inhibition might involve mTOR inhibition or stem from the alteration of the PI3K/AKT/mTOR pathway. *Thus, the levels of AKT, phospho-AKT (as a measure of AKT activation), and mTOR were firstly determined by Western blotting. …*

both AKT and phosphorylated AKT (p-AKT) were decreased after PtoxDpt treatment, hinting that downregulation of p-AKT may stem from the downregulated AKT.

A similar trend for mTOR, a downstream target of AKT, was also observed, indicating that the metastasis and invasion inhibition correlated with downregulation of mTOR that led to lower abundances of mTORC1 and mTORC2 complexes. The quantitative analysis of the proteins is shown in Figure 10(b); clearly, PtoxDpt-induced downregulation of both AKT and mTOR had significant statistical significance (p < 0 05 or 0.01).

On the other hand, in addition to migration and invasion inhibition, PtoxDpt also inhibited EMT, whether the PI3K/AKT/mTOR pathway was similarly involved in the EMT inhibition. To this end, the level of markers of the epithelium and mesenchymal cells, as well as AKT/mTOR, in the absence or presence of AKT inhibitor, LY294002, was further determined by Western blotting. …

[5] From Li et al: *Epithelial-mesenchymal transition (EMT) involves metastasis and drug resistance; thus, a new EMT reversing agent is required. It has shown that wild-type p53 can reverse EMT back to epithelial characteristics, and iron chelator acting as a p53 inducer has been demonstrated. Moreover, recent study revealed that etoposide could also inhibit EMT. Therefore, combination of etoposide with iron chelator might achieve better inhibition of EMT. To this end, we prepared* ***di-2-pyridineketone hydrazone dithiocarbamate S-propionate podophyllotoxin ester (PtoxDpt)*** *that combined the* ***podophyllotoxin (Ptox)*** *structural unit (etoposide) with the* ***dithiocarbamate unit (iron chelator)*** *through the hybridization strategy. The resulting PtoxDpt inherited characteristics from parent structural units, acting as both the p53 inducer and topoisomerase II inhibitor. In addition, the* ***PtoxDpt exhibited significant inhibition in migration and invasion,*** *which correlated with downregulation of matrix metalloproteinase (MMP). More importantly,* ***PtoxDpt could inhibit EMT in the absence or presence of TGF-β1****, concomitant to the ROS production, and the additional evidence revealed that PtoxDpt downregulated AKT/mTOR through upregulation of p53, indicating that* ***PtoxDpt induced EMT inhibition through the p53/PI3K/AKT/mTOR pathway****.*

both PtoxDpt and LY294002 downregulated vimentin (snail and slug) and contrarily upregulated E-cadherin, indicating that they acted in a similar way in EMT inhibition. Moreover, PtoxDpt also downregulated AKT and mTOR as LY294002 did, indicating that PtoxDpt-induced EMT inhibition involved the PI3K/AKT/mTOR pathway.

5.2 COLON

Colon cancers are generally slow growing and resectable if found early allowing for complete elimination. However, once they have begun to spread they are generally lethal.

As Zhang et al noted:

Colon cancer is one of the most common and lethal malignancies worldwide. Despite major advances in the treatment of colon cancer, the prognosis remains very poor. Thus, novel and effective therapies for colon cancer are urgently needed.

In the present study, the expression status of miR-218 and the role of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway were investigated in colon cancer samples. Firstly, we observed that miR-218 expression was significantly reduced, while PI3K/Akt/mTOR pathway activity was enhanced. The overexpression of miR-218 suppressed the proliferation, migration and invasion of LoVo colon cancer cells, whereas the inhibition of miR-218 promoted these processes.

Furthermore, the PI3K/Akt/mTOR signaling pathway was identified as a direct target of miR-218. The upregulation of miR-218 inhibited the activation of the PI3K/Akt/mTOR signaling pathway, as well as the expression of matrix metalloproteinase (MMP). The downregulation of miR-218 activated the PI3K/Akt/mTOR signaling pathway and promoted MMP9 expression. Taken together, our results demonstrate that miR-218 suppresses the proliferation, migration and invasion of LoVo colon cancer cells by targeting the PI3K/Akt/mTOR signaling pathway and MMP9. ***Our data indicate that miR-218 is a potential target in the treatment of colon cancer***

In this case above we have an interesting introduction of miRNA interactions which we will see becomes more common across a wide base of malignancies. Now Francipane and Lagasse note:

Classically, Akt has been viewed as the main upstream activator of mammalian target of rapamycin (mTOR). Indeed, activated Akt phosphorylates and inhibits tuberous sclerosis 2 (TSC2), allowing Ras homolog enriched in brain (Rheb) to accumulate in the GTP-bound state and trigger activation of the mTOR complex1 (mTORC1) pathway. mTORC1 is composed by mTOR, regulatory associated protein of mTOR (Raptor), mLST8/G-protein β-subunit like protein (GβL), RAS40 and Deptor.

The activation of mTOR in mTORC1 leads to phosphorylation of ribosomal S6 protein kinase 1 (S6K1) and eIF4E-binding protein 1 (4E-BP1), mediators of protein translation and cell growth [1]. mTOR response to a wide range of intracellular (energy and stress) and extracellular (nutrients, growth factors, hormones) signals is mediated through these effectors.

In response to nutrient and growth factor availability, mTORC1 suppresses autophagy, a process by which metabolically stressed cells recycle cytoplasmic components including organelles, to recover energy necessary for their survival. mTORC1 has also been recently identified as orchestrating anabolic cell growth by stimulating nucleotide synthesis through the pyrimidine synthesis pathway. Different from mTORC1, mTORC2 is composed of mTOR, rapamycin-insensitive companion of mTOR (Rictor), mLST8/GβL, stress-activated-protein-kinase interacting protein 1 (Sin1), proline-rich repeat protein-5 (PRR-5)/protein observed with Rictor-1 (Protor-1), and Deptor.

The upstream regulation of mTORC2 is not well defined, although ribosome association appears to be a major, if not the sole, mechanism of mTORC2 activation. mTORC2 plays an important role in cell survival, metabolism, proliferation and cytoskeleton organization, as it phosphorylates Protein Kinase Cα (PKCα), Serum/ glucocorticoid-regulated kinase 1 (SGK1), as well as Akt, allowing for complete activation of Akt. ***Akt is therefore both an upstream activator of mTORC1 and downstream effector of mTORC2****….*

Although mTOR is frequently activated in human cancers, mutation of the mTOR gene has been found only occasionally. This means that over-activation of the mTOR pathway is mostly due to signaling defects upstream of mTOR in the phosphatidylinositol-3-kinase (PI3K)/Akt/mTOR pathway. Mutations in PI3K alpha catalytic subunit kinase domain (PIK3CA) generally arise late in tumorigenesis, and can be identified in 32% of CRC tumors.

The above is a compelling statement. Namely mTOR participates but not because of any change in mTOR but due to mTORs key role in effecting so many other properties.

Loss of heterozygosity (LOH) and mutations in Phosphatase and tensin homolog (PTEN), a negative regulator of PI3K activity, have also been reported in CRC. Both PIK3CA mutations and PTEN loss lead to mTOR over-activation. Although mutations in Akt genes are rarely found in CRC, a somatic missense mutation of Akt1 (E17K) in the pleckstrin homology (PH) domain resulting in constitutive association of Akt1 with the plasma membrane and Akt1 prolonged activation has been reported in CRC, which can lead to mTOR deregulation [15].

Similarly, although rare, germline TSC gene mutations, which have been associated with colonic hamartomatous polyps, account for 1% CRC, possibly through mTOR pathway triggering…

mTORC1 is a major sensor of the organismal nutritional state. Indeed, caloric restriction lowers mTORC1 signaling in Paneth cells, a key constituent of the mammalian intestinal stem-cell (ISC) niche.

The starvation process is one way cells have to mitigate against malignancies. However the classic Warburg effect may take over and do a work around[6].

Paneth cells, in turn, stimulate small-intestinal stem cells to proliferate. Thus, mTOR inhibition can improve intestinal regeneration in patients affected by intestinal atrophy. While mTORC1

[6] https://www.researchgate.net/publication/322437754_Glucose_Warburg_Cancer_and_Pathways

inhibition can increase the number and regenerative capacity of ISCs, excessive mTORC1 stimulation can lead to the onset of cancer. Indeed, the importance of mTORC1 pathway in intestinal polyp formation has been well described in several papers using the Apc∆716 mice, a mouse model of familial adenomatous polyposis (FAP).

Through these studies, mTORC1 was found to stimulate chromosomal instability (CIN) through anaphase bridge formation, enhancing, as a consequence, both tumor initiation and progression. Similarly, ex vivo immunohistochemical studies on human colorectal adenomas and cancers confirmed that mTORC1 signaling occurs as an early event in the process of tumorigenesis, and participates in the progression of normal cells to a neoplastic phenotype, sustaining the bases of mTORC1-targeted drug development for therapy and prevention of colon polyps and cancers. Accordingly, Everolimus-mediated mTORC1 inhibition suppressed polyp formation and reduced mortality in Apc∆716 mice.

However, blocking of a specific pathway may disrupt the balance between signaling pathways and enhance oncogenic signals. In that regard, in parallel with its cytostatic effect, mTORC1 inhibition by Rapamycin strongly increased MAPK kinase (MEK)/ERK activity, resulting in the appearance of a spindle morphology and higher invasiveness of KRAS transformed intestinal epithelial cells

The last statement above is a worthy speculation. mTOR pathways almost always are aberrant not because of mTOR but due to other elements in the path. Thus perhaps the target should be against that aberrant element.

5.3 BREAST

Breast cancer is one of the most common cancers in women. As Hu et al have noted:

MicroRNAs (miRNAs) play an important role in human tumorigenesis as oncogenes or tumor suppressors. miR-99a has been reported as a tumor suppressor gene in various cancers in humans. However, only limited information about the function of miR-99a in human breast cancers is available. Here we investigated the expression of miR-99a in breast cancer tissue specimens and its antitumor activity in breast cancer cells.

We initially identified that the expression of miR-99a was significantly reduced in four breast cancer cell lines. More importantly, we found downregulation of miR-99a in breast cancer specimens from ten different patients. We then analyzed the mechanism of miR-99a in inhibiting tumorigenesis. Cell based assays that showed overexpression of miR-99a not only reduced breast cancer cell viability by inducing accumulation of cells at sub-G1 phase and cell apoptosis, but also inhibited tumorigenicity in vivo.

As a critical miR-99a target, we have shown that the function of mammalian target of rapamycin (mTOR) was greatly inhibited by miR-99a-based Luciferase report assay; overexpression of miR-99a reduced the expression of mTOR and its downstream phosphorylated proteins (p- 4E-BP1 and p-S6K1).

Again we see the interaction of a miRNA and mTOR.

Similar to restoring miR-99a expression, mTOR downregulation suppressed cell viability and increased cell apoptosis, whereas restoration of mTOR expression significantly reversed the inhibitory effects of miR-99a on the mTOR/ p-4E-BP1/p-S6K1 signal pathway and the miR-99a antitumor activity. In clinical specimens and cell lines, mTOR was commonly overexpressed and its protein levels were statistically inversely correlated with miR-99a expression. Taken together, these results have demonstrated that miR-99a antitumor activity is achieved by targeting the mTOR/p-4E-BP1/pS6K1 pathway in human breast cancer cells. This study suggests a potential therapeutic strategy to effectively control breast cancer development….

MTOR is a direct target of miR-99a in breast cancer cells…

Our study has further confirmed that miR-99a is a tumor suppressor gene, which is commonly downregulated in both breast cancer clinic tissues and breast cancer cell lines. Overexpression of miR-99a reduces breast cancer cell viability, induces apoptosis and inhibits tumorigenicity in vitro and in vivo through targeting mTOR/p-4E-BP1/p-S6K1 pathway. mTOR plays a critical role in mediating miR-99a dependent biological functions in breast cancer. miR-99a/mTOR might therefore be used as potential therapeutic targets in breast cancer.

5.4 BLADDER

Bladder cancer can be seen in two forms; noninvasive and invasive[7]. As Puzio-Kuter et al have noted:

Although bladder cancer represents a serious health problem worldwide, relevant mouse models for investigating disease progression or therapeutic targets have been lacking. We show that combined deletion of p53 and Pten in bladder epithelium leads to invasive cancer in a novel mouse model. Inactivation of p53 and PTEN promotes tumorigenesis in human bladder cells and is correlated with poor survival in human tumors.

Furthermore, the synergistic effects of p53 and Pten deletion are mediated by deregulation of mammalian target of rapamycin (mTOR) signaling, consistent with the ability of rapamycin to block bladder tumorigenesis in preclinical studies. Our integrated analyses of mouse and human bladder cancer provide a rationale for investigating mTOR inhibition for treatment of patients with invasive disease…

Finally, our findings provide a strong rationale for therapeutic targeting of the mTOR signaling pathway in invasive bladder cancer, particularly in patients having deregulated p53 and PTEN, as well as a preclinical model for effective assessment of appropriate therapies. Notably, clinical successes with rapamycin analogs as single agents have been limited, with some notable exceptions. In contrast, rapamycin analogs have been shown to be more promising in

[7] https://www.researchgate.net/publication/336460663_Bladder_Cancer_An_Interesting_Set_of_Diagnostic_Options

combination with standard chemotherapy or agents that target other signaling pathways, particularly the RAS/MEK/MAP kinase pathway.

Therefore, while rapamycin analogs represent a promising strategy in the treatment of invasive bladder cancer, it will also be important to investigate their efficacy in combination with other targeted therapies and/or chemotherapy. Notably, our study provides a relevant preclinical model for investigating targeted therapies for invasive bladder cancer, particularly those based on perturbation of mTOR signaling.

As Xu et al (2013) have noted:

miRNAs are involved in cancer development and progression, acting as tumor suppressors or oncogenes. In this study, miRNA profiling was conducted on 10 paired bladder cancer tissues using 20 GeneChip miRNA Array, and 10 differentially expressed miRNAs were identified in bladder cancer and adjacent noncancerous tissues of any disease stage/grade. After being validated on expanded cohort of 67 paired bladder cancer tissues and 10 human bladder cancer cell lines by quantitative real-time PCR (qRT-PCR), it was found that miR-100 was downregulated most significantly in cancer tissues.

Ectopic restoration of miR-100 expression in bladder cancer cells suppressed cell proliferation and motility, induced cell-cycle arrest in vitro, and inhibited tumorigenesis in vivo both in subcutaneous and in intravesical passage. Bioinformatic analysis showed that the mTOR gene was a direct target of miR-100. siRNA-mediated mTOR knockdown phenocopied the effect of miR-100 in bladder cancer cell lines. In addition, the cancerous metastatic nude mouse model established on the basis of primary bladder cancer cell lines suggested that miR-100/mTOR regulated cell motility and was associated with tumor metastasis.

Both mTOR and p70S6K (downstream messenger) presented higher expression levels in distant metastatic foci such as in liver and kidney metastases than in primary tumor. Taken together, miR-100 may act as a tumor suppressor in bladder cancer, and reintroduction of this mature miRNA into tumor tissue may prove to be a therapeutic strategy by reducing the expression of target genes.

More recently Xu et al (2019) have noted:

Yes-associated protein 1 (YAP1[8]) and mammalian target of rapamycin (mTOR) signaling pathways have been found to be deregulated in bladder cancer and accelerate the malignant progression of bladder cancer. However, the crosstalk between YAP1 and mTOR and its role in bladder cancer progression remains unclear.

[8] See NCBI, https://www.ncbi.nlm.nih.gov/gene/10413 *This gene encodes a downstream nuclear effector of the Hippo signaling pathway which is involved in development, growth, repair, and homeostasis. This gene is known to play a role in the development and progression of multiple cancers as a transcriptional regulator of this signaling pathway and may function as a potential target for cancer treatment. Alternative splicing results in multiple transcript variants encoding different isoforms.*

The aim of the present study was to investigate this crosstalk and the results revealed that the expression of YAP1 and mTOR was elevated in bladder cancer tissues compared with that in adjacent normal tissues.

Knockdown of either mTOR or YAP1 with siRNA transfection significantly repressed the proliferation ability and induced apoptosis of HT-1376 and J82 bladder cancer cells, particularly when YAP1 and mTOR were downregulated simultaneously. Upregulation of mTOR increased the mRNA and protein levels of YAP1 and enhanced its nuclear accumulation.

In turn, YAP1 upregulation increased mTOR expression, reduced its protein degradation and increased its stability. In addition, immunofluorescence and Duolink assays demonstrated that YAP1 and mTOR were co-localized in the nucleus. Immunoprecipitation assay demonstrated that the YAP1 protein was able to bind to the mTOR protein.

Moreover, YAP1 combined with S-phase kinase-associated protein 2 (SKP2) and positively regulated its expression. Furthermore, the promotion of cell growth and inhibition of cell apoptosis induced by YAP1 overexpression were abolished when SKP2 was downregulated in HT-1376 and J82 cells. Taken together, the findings of the present study indicated that the crosstalk between YAP1 and mTOR plays a pivotal role in accelerating the progression of bladder cancer, which may provide new insights into the role of the YAP1/mTOR axis in the occurrence and development of bladder cancer.

5.5 RENAL

Renal cancer is often see in patients with T2 diabetes and obesity. As such it is suspected to be driven by reactive oxygen species as well as the pathways affected by metabolic stress. As Tian et al note:

RCC is regarded as one of the most lethal cancers because of the rare available therapies and lack of proper diagnosis biomarkers at early stages. RCC is mainly classified as clear cell renal cell carcinoma (ccRCC, 85%), papillary renal cell carcinoma (PRCC, 0–15%), chromophobe renal cell carcinoma (chRCC, 5%) and collecting duct carcinoma and medullary carcinoma (1%). Generally, mTOR signaling regulates cell metabolism, and RCC is also a cancer of metabolism dysregulation.

Data from TCGA on a ccRCC study in 2013 demonstrated genetic alterations in components of each level of the PI3K/Akt signaling pathway cascade (PIK3CA, PIK3R1, PIK3R2, PTEN, PDPK1, AKT1, AKT2, AKT3, FOXO1, FOXO3, MTOR, RICTOR, TSC1, TSC2, RHEB, AKT1S1, and PRTOR), mainly including GNB2L1 amplification (6%), PI3KCA amplifications or mutations (5%), PTEN deletions or mutations (5%) or MTOR mutations (6%). Clustered MTOR mutations, as well as mutations in AKT1, AKT3 and RHEB, contributed to PI3K/Akt and mTOR hyperactivation in ccRCC.

In addition, the cross talk between VHL/HIF and the PI3K/Akt pathway via a positive feedback mechanism contributes to the sustaining activation of PI3K/Akt signaling in ccRCC. The rate of genetic alterations in PI3K/Akt pathway components in pRCC is 28% according to the TCGA

database, including mutations of PTEN and PI3K subunits and amplifications of GNB2L1, PDK1 and RPTOR amplifications. In chRCC, PTEN was mutated most frequently which occurred in 11% of patients, and mutations of AKT1, TSC1/TSC2 and mTOR in the mTOR signaling pathway have also been shown

5.6 THYROID

Thyroid cancers fall into a multiplicity of types, most being relatively benign in character[9]. However anaplastic thyroid cancer is one of the most aggressive cancers with survival limited to several months at best. It is a very rare form but its aggressive behavior is dispositive of mTOR loss of control.

As Wagle et al have noted:

Everolimus is a Food and Drug Administration–approved oral allosteric inhibitor of mTOR. Tumors that exhibit a dependency on the mTOR pathway might have enhanced sensitivity to mTOR inhibition. Inactivating mutations in the tumor-suppressor genes TSC1[10], TSC2[11], and STK11[12] result in mTOR-pathway activation and are targetable by TOR inhibitors in hamartoma syndromes and in malignant perivascular epithelioid-cell tumors. In a phase 2 study of everolimus in urothelial carcinoma, whole-genome sequencing in a patient who had a durable complete remission revealed a somatic TSC1 mutation.

We recently identified an additional mechanism of exquisite sensitivity to everolimus in a patient with metastatic urothelial carcinoma: activating mutations in mTOR itself. Although mechanisms of sensitivity to everolimus are beginning to be identified, mechanisms of clinically acquired resistance to everolimus remain unknown.

This report of a nonsense mutation in TSC2 in a tumor from a patient with anaplastic thyroid cancer who had a response to everolimus supports the notion that cancers of diverse types with mTORpathway–activating mutations are sensitive to mTOR inhibitors. Once resistance emerged

[9] https://www.researchgate.net/publication/331935614_Thyroid_Cancer_Seek_and_Ye_Shall_Find

[10] See NCBI https://www.ncbi.nlm.nih.gov/gene/7248 *This gene is a tumor suppressor gene that encodes the growth inhibitory protein hamartin. The encoded protein interacts with and stabilizes the GTPase activating protein tuberin. This hamartin-tuberin complex negatively regulates mammalian target of rapamycin complex 1 (mTORC1) signalling which is a major regulator of anabolic cell growth. This protein also functions as a co-chaperone for Hsp90 that inhibits its ATPase activity. This protein functions as a facilitator of Hsp90-mediated folding of kinase and non-kinase clients, including Tsc2 and thereby preventing their ubiquitination and proteasomal degradation.*

[11] See NCBI https://www.ncbi.nlm.nih.gov/gene/7249 *Mutations in this gene lead to tuberous sclerosis complex. Its gene product is believed to be a tumor suppressor and is able to stimulate specific GTPases. The protein associates with hamartin in a cytosolic complex, possibly acting as a chaperone for hamartin. Alternative splicing results in multiple transcript variants encoding different isoforms*

[12] See NCBI https://www.ncbi.nlm.nih.gov/gene/6794 *This gene, which encodes a member of the serine/threonine kinase family, regulates cell polarity and functions as a tumor suppressor. Mutations in this gene have been associated with Peutz-Jeghers syndrome, an autosomal dominant disorder characterized by the growth of polyps in the gastrointestinal tract, pigmented macules on the skin and mouth, and other neoplasms. Alternate transcriptional splice variants of this gene have been observed but have not been thoroughly characterized.*

in this patient, the mechanism of acquired resistance to everolimus was identified as a mutation in mTOR that prevented everolimus from binding to mTOR.

The mutant mTOR remained sensitive to direct mTOR kinase inhibition. Anaplastic thyroid cancer is a highly aggressive and rapidly fatal disease, for which there is no adequate treatment. To date, limited molecular profiling studies in this disease have identified a few targetable alterations. Oncogenic BRAF mutations have been described, and a response to the RAF inhibitor vemurafenib was observed in a patient with BRAF-mutant anaplastic thyroid cancer. Additional targetable alterations include mutations in PIK3CA, PTEN, KRAS, NRAS, and ALK.

Genomic profiling of anaplastic thyroid cancer to screen for mTOR-pathway alterations may identify subgroups of patients who are potential candidates for enrollment in clinical trials of mTOR-directed therapies.

Indeed, several activating alterations in the mTOR pathway — including alterations in MTOR, TSC1 or TSC2, STK11, and RHEB — have been observed recurrently in multiple cancer types, providing the rationale for the development of so-called basket trials of mTOR inhibitors in patients with diverse tumor types who have somatic mTORpathway alterations. Although cancers driven by a dominant oncogene frequently have dramatic responses to targeted kinase inhibitors, the tumors invariably become resistant to these agents.

The majority of known mechanisms of clinical resistance involve secondary mutations in the target kinase. Such mutations has been described for ABL, KIT, EGFR, ALK, BRAF, MEK, PDGFRA, FLT3, and ROS1. Indeed, in the case of every kinase inhibitor for which a resistance mechanism has been described, there is clinical evidence that resistance can occur through a secondary alteration in the target kinase.

Our results indicate that acquired resistance to mTOR inhibition can occur through the same mechanism. It is likely that additional secondary mutations in the mTOR FRB domain will be identified in patients, as suggested by studies in fission yeast and experience with other tumor types. We speculate that these patients would be ideal candidates for clinical trials of mTOR kinase inhibitors.

5.7 PROSTATE

Prostate cancer is the most frequent cancer for men in the US[13]. As Tian et al note:

The mTOR pathway is reported to be significantly active in prostate cancer. The PI3K/Akt pathway is found aberrant in PCa cell lines, xenograft models, and 30–50% primary PCa tissue samples. *Genetic alterations of the mTOR pathway were detected in 42% of primary prostate tumors and all metastatic tumors. Aberrant PTEN/Akt expression was found in 42% of PCa tissues.*

Again it is the mTOR pathway and not mTOR itself.

[13] https://www.researchgate.net/publication/264960277_Prostate_Cancer_A_Systems_Approach

As PTEN loss was demonstrated to be associated with a high Gleason score, PCa pathological stages and promoted the progression of lymph node metastasis, PTEN may serve as a potential early prognostic marker in prostate cancers. High levels of phosphorylated-4EBP1 and eIF-4E are significantly related to increased mortality in PCa patients, implying that downstream effectors of the mTOR pathway may be a potential prognostic indicator for PCa progression.

Studies in PCa cell lines indicated that the PI3K/Akt/mTOR pathway contributed to PCa radio-resistance (RR) through mechanisms of intrinsic radio-resistance, cancer cell proliferation and hypoxia, and in those PCa RR cell lines, the PI3K/Akt/mTOR pathway was the most active. Moreover, activation of the PI3K/Akt/mTOR pathway was also reported to be involved in epithelial mesenchymal transition (EMT) and cancer stem cells (CSCs) in prostate cancer radio-resistance

5.8 GASTRIC

Gastric cancer is a declining cancer but if has regional presence due to diet. As Tian et al note:

Researches demonstrated that PIK3CA, PIK3CB, AKT1 and mTOR are overexpressed in GC cell lines, and mTOR pathway is active in almost 60% of gastric cancer patients. PIK3CA is reported to be commonly mutated and amplified at frequencies of around 18% and 5%, respectively. Three mutation hotspots that exist in almost 80% of PIK3CA mutations are E545K (exon 9), E542K (exon 9) and H1047R (exon 20).

As reported, PIK3CA mutation frequency in gastric cancer is associated with cancer stage and Epstein–Barr virus (EBV) infection. PTEN, which is a key inhibitor of the PI3K pathway, is a significant tumor suppressor gene. According to the TCGA database of gastric cancer, deletion, mutation and amplification of PTEN each occur in 0.3%, 3.1% and 4% of cases, respectively.

The alteration frequency of PIK3CA and PTEN varies significantly in different populations: for example, between Asian and Caucasian GC patients, the rate is 7% compared to 15% for PIK3CA mutations, 21% compared to 4% for PTEN deletion, and 47% compared to 78% for PTEN loss, respectively.

Another research found 19% PTEN mutations in GC patients in a Chinese population, including missense, nonsense, deletion, and mutations in intron 6. PTEN tends to be mutated more frequently in advanced stage or less differentiated GC. Despite AKT overexpression in 74% of GC patients examined by immunochemistry, the genetic alterations in AKT are very few at approximately 1% to 3% in GC. Although the exact genomic changes that occur in mTOR signaling downstream of PI3K/Akt are not well clarified, it is reported that phosphorylated-mTOR overexpression is related to some clinicopathological features and poor prognosis in GC patients alone or combined with TSC1 downregulation.

In an Eastern Chinese population, mTORC1 polymorphisms contribute to the risk of GC. Moreover, an immunohistochemical study via GC tissue microarray demonstrated that aberrant S6K1 expression may lead to cancer initiation, invasion and metastasis of GC

5.9 LUNG

We have seen a proliferation of immunotherapeutic approaches to lung cancers, especially NSCLC. As Tian et al note:

In non-small cell carcinomas (NSCLC), PI3K pathway activation is found in 50–70% of patients with AKT phosphorylation. Mutations in EGFR, Kirsten rat sarcoma viral oncogene (KRAS), PI3K, amplification of PIK3CA and loss of PTEN can lead to PI3K pathway activation. As reported by The Cancer Genome Atlas (TCGA) Research Group, alterations in the PI3K/Akt pathway, which is upstream of mTOR signaling, were detected in 47% of squamous cancers (including PIK3CA alterations in 16%, PTEN alterations in 15%, AKT3 alterations in 16%, AKT2 alterations in 4% and AKT1 alterations < 1% of the total samples).

Actually, genomic amplification is much more frequent than somatic mutations in PI3KCA in lung cancers. In addition, PI3KCA was found to have copy number amplifications in 33% of squamous cell lung carcinomas, which occurred independently of the PI3KCA gene mutation, demonstrating that each event is probably sufficient to initiate tumorigenesis.

Besides, in a report of 51 Japanese small cell lung carcinoma (SCLC) patients, 36% of the tumors had genetic mutations related with mTOR pathway. Phosphorylated mTOR is demonstrated to contribute to SCLC progression…

Again and again we see that the mTOR targets are really targeting other pathway elements.

5.10 MELANOMA

Melanoma is the deadliest form of skin cancer[14]. As Kong et al note:

mTOR is a serine and threonine protein kinase and plays crucial roles in transcriptional regulation, initiation of protein synthesis, ribosome biogenesis, metabolism and apoptosis, etc., after being activated by various factors. mTOR signaling pathway has been the key targets for cancer treatment. Inhibitors for mTOR, including rapamycin and the derivatives RAD001, CCI-779, and AP23573, usually bind FKBP12, inhibit tumor growth, and even induce apoptosis. Unfortunately, clinical trials using mTOR inhibitors in melanoma patients are not successful.

A phase II clinical trial using RAD001 in 24 cases of metastatic melanoma showed that the median progression-free survival (PFS) was about 3 months. Another phase II clinical trial using RAD001 in combination with temozolomide in 48 patients with advanced stage IV unresectable melanomas showed that the median PFS was only 2.4 months.

[14] https://www.researchgate.net/publication/264960157_Melanoma_Genomics
and
https://www.researchgate.net/publication/325106051_mi_RNA_and_Melanoma

Genetic selection of specific target may be useful for the establishment of therapeutic strategy for advanced melanoma patients. The two clinical trials using mTOR inhibitors have not screened the genetic mTOR nonsynonymous mutations (termed as mutation in this study if not specified) in the melanoma patients.

Therefore, it remains to be determined whether melanoma patients bearing certain genetic mutations in mTOR will respond better to mTOR inhibitors. …

Because mTOR spans 58 exons and mTOR protein is composed of 2,549 amino acids, it is difficult to analyze the genetic aberrations of mTOR and the functional consequences of mTOR nonsynonymous mutations.

Currently, the gene aberration data of mTOR mainly come from genomic sequencing or exome sequencing of cancer samples. However, these data have not been validated. More importantly, due to the difference of predominant melanoma subtypes between Caucasians (mainly cutaneous melanomas) and Asians, mTOR aberrations in acral and mucosal melanomas remain largely unknown.

Melanoma is currently treated by immunotherapeutic methods with some success. However as with many of these approaches, success may be presented in say 40% of the cases and yet 60% either do not respond or stay in remission. Thus it is likely that multi-regimen approaches using mTOR targets may prove efficacious.

6 THERAPEUTIC OPTIONS

If mTOR and its conjugates are so critical to normal cell homeostasis then aberrant behavior may be mitigated therapeutically via control using rapamycin analogs.

As Boutouja, et al, have noted:

After the discovery of Rapamycin, this macrolide compound was used as an antifungal drug against infections with Aspergillus fumigatus, Candida albicans and Cryptococcus neoformans. When the important functional role of mTOR became evident during the basic research work with rapamycin, the combination of rapamycin with cyclosporine A was established as an important immunosuppressant against transplant rejection because of the inhibition of T-cell proliferation.

Moreover, based on its cytostatic activity, rapamycin could be used as an anti-cancer agent. More recently, rapamycin has also shown to contribute to the prevention of coronary artery restenosis as well as to the treatment of neurodegenerative diseases. New inhibitors of mTOR are being designed.

6.1 FIRST GENERATION

We start with the classic first generation as follows:

*The so called **first generation** of mTOR inhibitors comprises the natural compound rapamycin (generic name: Sirolimus) and its engineered derivates, the so called rapalogs. They have in common that they also bind to FKBP12, but they are supposed to have context-dependent and more favorable pharmacokinetic profile when compared to rapamycin. Temsirolimus (Torisel) is the prodrug of rapamycin and is often used against renal cell carcinoma.*

Everolimus is a rapalog that is used in transplantation medicine under the names Zortress or Certican, as well as in oncology for general tumor under the names Afinitor or Biocon. The second generation of inhibitors targets both TORC1 as well as TORC2 by competing with ATP at the catalytic site of the mTOR kinase, which is present in both complexes. Similar to rapalogs, they can decrease protein translation and attenuate cell proliferation in several cancer cell lines.

6.2 SECOND GENERATION

The second generation inhibitors are described below:

*Along with the directly kinase-dependent functions of mTOR, the **second generation** inhibitors also block the feedback activation of the PI3K and AKT signaling pathways. Therefore, in addition to the optimized inhibition of TORC1 in rapamycin-resistant cell lines, these inhibitors are thought to block TORC2 as well as interfere with the interplay with the PI3K and AKT.*

Another approach is to target mTOR associated proteins, like the inhibition of RHEB by the small molecule NR1, which inhibits TORC1-dependent phosphorylation of S6K1.

6.3 THIRD GENERATION

Finally the current third generation mTOR inhibitors are described as follows:

*The **third generation** of mTOR inhibitors is supposed to be used in cells that have developed a resistance against both first- and second generation inhibitors. Theses inhibitors are bivalent molecules that exploit the juxtaposition of the corresponding two drug-binding pockets. RapaLink-1 consists of a rapamycin-FRB compound linked to the mTOR kinase inhibitor TORKi. Therefore, exploitation of both the kinase domain as well as FRB domain of mTOR should potentially inhibit mTOR-related dysfunctions in the context of tumor growth. Moreover, the methodological approach to design novel bivalent inhibitors could be applied to resistances in other disease-relevant signaling pathways.*

6.4 SUMMARY

We now provide a summary of these therapeutics. As Yuan et al have noted:

The mammalian target of rapamycin (mTOR) is an intracellular serine/threonine protein kinase positioned at a central point in a variety of cellular signaling cascades. The established involvement of mTOR activity in the cellular processes that contribute to the development and progression of cancer has identified mTOR as a major link in tumorigenesis.

Consequently, inhibitors of mTOR, including temsirolimus, everolimus, and ridaforolimus (formerly deforolimus) have been developed and assessed for their safety and efficacy in patients with cancer. Temsirolimus is an intravenously administered agent approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) for the treatment of advanced renal cell carcinoma (RCC).

Everolimus is an oral agent that has recently obtained US FDA and EMEA approval for the treatment of advanced RCC after failure of treatment with sunitinib or sorafenib. Ridaforolimus is not yet approved for any indication. The use of mTOR inhibitors, either alone or in combination with other anticancer agents, has the potential to provide anticancer activity in numerous tumor types.

Cancer types in which these agents are under evaluation include neuroendocrine tumors, breast cancer, leukemia, lymphoma, hepatocellular carcinoma, gastric cancer, pancreatic cancer, sarcoma, endometrial cancer, and non-small-cell lung cancer. The results of ongoing clinical trials with mTOR inhibitors, as single agents and in combination regimens, will better define their activity in cancer

Following upon the previous discussion we have from As Conciatori et al who note:

Currently, two rapalogs, temsirolimus and everolimus, are approved in US and EU for the treatment of different types of cancer. A third agent—ridaforolimus, was tested as maintenance therapy in sarcoma patients achieving disease control with chemotherapy…

Conversely, temsirolimus monotherapy is approved for the first-line treatment of metastatic RCC (mRCC) with poor risk features based on the results of the ARCC trial, and for the treatment of relapsed/refractory Mantle Cell Lymphoma (MCL); everolimus monotherapy, on the other hand, is approved for the treatment of advanced, pretreated mRCC and progressive Neuroendocrine Tumors (NET) of Gastro-Entero-Pancreatic (GEP) and lung origin, based on the RADIANT-3 and -4 trials.

Rapalogs have been extensively evaluated for the treatment of many other tumor types, but such investigations have met with very limited clinical success, despite the fact that the PI3K/AKT/mTOR pathway is frequently dysregulated in human cancers and it plays a fundamental biological role as a master regulator of cell growth and proliferation, cellular metabolism, and cell survival.

This might be due, at least in part, to the fact that clinical trials with mTOR inhibitors have been conducted in unselected patient populations, without enrichment for potential biomarkers of sensitivity or predictors of clinical activity. Rapalogs monotherapy is in general well tolerated and adverse events are manageable and related to their mechanism of action; their description is beyond the scope of this review, but are well described in the literature mTOR Kinase Inhibitors The limited clinical success of rapalogs may be related to feedback loops involved in cell survival responses.

For instance, under normal conditions, mTORC1 activates p70S6K1 which phosphorylates IRS, thereby leading to its degradation and downregulation of PI3K signaling: rapamycin treatment blocks mTORC1 activity, IRS is activated and PI3K signaling is upregulated. Rapalogs can also cause activation of AKT through disruption of a negative feedback loop on the mTORC2 complex, which is involved in cancer cell growth and survival: this limitation led to development of a second generation of mTOR inhibitors, which are ATP-competitive mTOR kinase inhibitors.

These compounds suppress the activation of both mTORC1 and mTORC2, thus completely blocking PI3K/AKT signaling and prevent the feedback activation of AKT after treatment with rapalogs. Due to the sequence similarity between mTOR and PI3K, particularly at the kinase active sites, ATP-competitive inhibitors often inhibit both PI3K and mTOR activity and are therefore referred to as dual inhibitors.

Again this demonstrates the secondary albeit controlling effects of mTOR and its complexes. It appears that any therapeutic targeting mTOR would be in effect targeting deficits in other pathway elements.

Several dual PI3K/mTOR inhibitors (e.g., GDC-0980, PF-04691502, BEZ235, XL765, GSK2126458) are currently being developed for clinical use, on the assumption that the vertical blockade of two different crucial nodes along the PI3K signaling pathway might result in more complete pathway inhibition, disruption of pathway-reactivating feedback loops, and eventually enhanced anti-tumor activity.

However, limited clinical experience obtained so far suggests that such agents have only modest single agent anti-tumor activity. This may partly be due to the narrow therapeutic window associated with these drugs that limits their dose escalation, or to the unselected populations of patients enrolled into these early phase studies.

Alternatively, clinically meaningful mTOR pathway blockade could be achieved by non-ATP-competitive allosteric modulators of protein functions and possibly by Hsp90 inhibitors.

Third-Generation mTOR Inhibitors Based on ev6.3. Third-Generation mTOR Inhibitors Based on evidence that mutations in either the FRB or the kinase domain can induce resistance to rapalogs and mTOR kinase inhibitors, third-generation mTOR inhibitors exploiting the unique juxtaposition of two drug (first- and second-generation mTOR kinase inhibitors)—binding pockets to create a bivalent interaction that allows inhibition of the mutants have been developed. Rapalink-1 is more potent than first- and second- generation mTOR inhibitors in reducing the levels of both p-4EBP1 and cell proliferation; as a consequence, RapaLink-1 led to regression of tumor xenografts models and could durably block mTORC1; moreover, it showed better efficacy than rapamycin or mTOR kinase inhibitors, potently blocking cancer-derived, activating mutants of mTOR.

No clinical data on third-generation mTOR inhibitors are available to date.

Potential Biomarkers of Sensitivity to mTOR Pathway Inhibitors Several preclinical studies have suggested that alterations in certain tumor suppressor genes (namely PTEN and TSC1/2), as well as mTORC1 phosphorylation sites on 4E-BP1 and p70S6K1 may be correlated with sensitivity or resistance to rapalogs.

Moreover, other markers of upstream and downstream signaling have been evaluated to help predict clinical responses. From a clinical point of view, a recent analysis of 39 patients with (n = 22) or without (n = 17) exceptional clinical benefit from everolimus treatment across various tumor types (13 gastric cancers, 15 RCCs, 2 thyroid cancers, 2 head and neck cancer, and 7 sarcomas) reported mutations in genes along the mTOR pathway (mTOR, TSC1, TSC2, Neurofibromin (NF) 1, PhosphIinositide-3-Kinase Catalytic (PIK3C) A and G) in 10/22 responder patients (45%), with mutations in mTOR, TSC1/2, and NF1 exclusively found in responders; conversely, recurrently mutated genes of Fibroblast Growth Factor Receptor (FGFR) 4 and BRCA1-Associated Protein (BAP) 1 were noted only in patients without clinical benefit

7 mTOR AND miRNAS

miRNAs have become a significant factor in understanding malignancies. Their number and their influences on a variety of malignancies has exploded in the past decade. The small single stranded RNA, about 22 bases in length, tend to act by interfering with normal genetic processes. They also have the ability to move from cell to cell and also to go through intra and extra vasation thus putatively facilitating metastatic processes.

miRNAs have exploded in terms of importance in a variety of cancers. They can be pro or anti-cancer drivers, they can be vehicles for metastatic activation at local and distant sites, they can be targets for identifying various elements in the blood, urine or otherwise. Making any single statement is often just the first step in recognizing the impact of miRNAs. We present just some typical examples.

7.1 COLON

We have previously demonstrated the impact of miRNAs on breast cancer modulation of mTOR. As Zhang et al have noted a similar effect on colon cancer as follows:

Colon cancer is one of the most common and lethal malignancies worldwide. Despite major advances in the treatment of colon cancer, the prognosis remains very poor.

Thus, novel and effective therapies for colon cancer are urgently needed. In the present study, the expression status of miR-218 and the role of the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway were investigated in colon cancer samples.

Firstly, we observed that miR-218 expression was significantly reduced, while PI3K/Akt/mTOR pathway activity was enhanced.

The overexpression of miR-218 suppressed the proliferation, migration and invasion of LoVo colon cancer cells, whereas the inhibition of miR-218 promoted these processes. Furthermore, the PI3K/Akt/mTOR signaling pathway was identified as a direct target of miR-218. The upregulation of miR-218 inhibited the activation of the PI3K/Akt/mTOR signaling pathway, as well as the expression of matrix metalloproteinase (MMP)9. The downregulation of miR-218 activated the PI3K/Akt/mTOR signaling pathway and promoted MMP9 expression.

Taken together, our results demonstrate that miR-218 suppresses the proliferation, migration and invasion of LoVo colon cancer cells by targeting the PI3K/Akt/mTOR signaling pathway and MMP9. Our data indicate that miR-218 is a potential target in the treatment of colon cancer

This is just one of hundreds of putative targets.

7.2 THYROID

In a similar manner, miRNAs impact mTOR in papillary thyroid cancers as noted by Mina et al:

In this study we identified a panel of deregulated miRNAs in PTC specimens compared to normal thyroid and among these we showed that miR-451a is underexpressed and displays tumor suppressor functions by targeting multiple elements of the AKT/mTOR pathway.

Our experimental strategy included: miRNA profiling, validation in an independent dataset from TCGA, literature review and meta-analysis, combined analysis of PTC clinical samples and in vitro cell models and functional studies…

Here we showed that miR-451a mimic reduces both the c-MYC protein levels and the activation of AKT pathway.

As activated AKT pathway directly controls c-MYC protein levels [52], we cannot exclude the possibility that the observed reduction of c-MYC protein may be due to the combined action of miR-451a not only on c-MYC but also on its upstream regulators, namely on AKT. Indeed, AKT (specifically AKT1) is a target of miR-451a (miRTarBase ID MIRT005740) and consistently with this notion, we showed its reduction following miR-451a transfection. AKT is a central mediator in the PI3K/AKT/mTOR pathway that in turn regulates fundamental cellular processes as proliferation and migration. Importantly, AKT is activated in many cancers, including thyroid carcinomas, where it is involved in tumor formation and progression.

Evidence of its activation has been reported also in PTC and a trend toward AKT1 overexpression, both at mRNA and protein level, has already been described. In line with these observations, we found a moderate but significant overexpression of AKT1 mRNA in PTC samples from TCGA…In biochemical analyses, consistently with AKT reduction, we observed decreased phosphorylation of its downstream effectors mTOR and ribosomal protein S6, indicative of reduced pathway activation.

Interestingly, we found also decreased expression of total S6 protein. However, this reduction may be due to feedback regulation rather than to a direct targeting by miR-451a. Indeed, S6 is not a reported target of miR-451a (according to miRTarBase v16) and we have previously showed its decrease following mTOR silencing. Thus, we hypothesize that miR-451a by targeting AKT indirectly impairs the downstream activation of mTOR and this in turn causes S6 protein reduction…

Collectively, our functional analyses showed that in PTC, miR-451a affects cell proliferation and migration and targets multiple elements of the AKT/mTOR pathway, thus appearing to play a role as tumor suppressor miRNA in this neoplasia. We are aware that here we focused primarily on selected targets of miR-451a, already validated in other experimental sets, and that the identified link miR-451a/AKT pathway may represent only one, among many, of the molecular mechanisms by which this miRNA exerts its functions. Additional and more in-depth studies are thus required to fully elucidate the biological role of miR-451a in PTC.

However, to our knowledge this is the first study investigating the functional role of miR- 451a in PTC and the identification of a link miR-451a/ AKT pathway in this tumor is noteworthy. Indeed, AKT pathway, along with MAPKs pathway, is a central hub in the signaling networks involved in thyroid carcinogenesis and several deregulated miRNAs have already been reported to target this pathway at multiple levels…

We believe that a comprehensive understanding of miRNAs and their impact is essential. mTOR and its complexes are just a small part of that study.

8 OBSERVATIONS

We now consider several observations regarding the mTOR effects and options.

8.1 DIAGNOSTIC AND PROGNOSTIC VALUE

mTOR and its complexes act normally when driven by their activators. The question of there being some mutated version of mTOR and/or its complexes which in turn make cells act in an overdrive manner is questionable. mTOR and its complexes are acting in a normal fashion, albeit in a manner that can exacerbate metastatic influences.

This then begs the question of looking at mTOR and its complexes as diagnostic or prognostic. Is their presence abnormal, or is it the excess which is the driver? One suspects we are not looking at a BRAF V600 situation or some other common alteration of genomic structure.

However, as we have noted based on research in various fields, mTOR and its complexes effect cancer via their activation by upstream or doing likewise via downstream. It appears that mTOR and its complexes per se are not dispositive.

8.2 CELLULAR DYNAMICS

We have always struggled with the physical/chemical dynamics of the proteins involved in the pathways we have been discussing. The pathway models are descriptive, yet they fail to account for such things as protein concentrations, reaction rates, and the like. They just show one molecule impacting on another. With mTOR and its complexes we see a prolific set of upstream and downstream molecules all at various concentrations, all with varying reaction rates. Thus to fully understand the process we must come to grips with these less than secondary factors. For example we know that mTOR and its complexes rely upon their production via translation in the cell, via the impact of promoters and the like. Understanding and explaining these complex interactions will be essential to using this mechanism effectively.

8.3 MARKERS

One form of markers would be the miRNAs which impact mTOR and its pathways. mTOR by itself does not appear to have any markers at this time.

8.4 MIRNA IMPACTS

It was shown briefly that miRNAs can work alongside mTOR and its complexes to effect a malignant state. miRNAs have exploded in size since they had been first identified. Some prevent malignant states, some promote, some transmit them. We see that miRNAs have had a significant role in many of the mTOR related cancers we have examined. Thus it is essential to understand and incorporate these in any reasonable approach to malignancies.

8.5 THERAPEUTIC TARGETING

Like many targets mTOR and its associates are attractive but do not act in a vacuum. Thus rapalogs may work from time to time but alternative paths may dominate and adaptive mutations may create new paths.

8.6 MUTATIONAL DYNAMICS

We have recently discussed mutational effects as drivers of metastatic development[15]. The effects are critical to understand as is the tumor associate macrophages[16], and tumor micro environment.

8.7 IMMUNE TARGETING

There is an ongoing search for markers which are targetable by the immune system so as to effect an immunotherapeutic approach to eliminating cancer cells. The question then is; does mTOR and/or its complexes effect such putative surface markers in a malignant cell?

As Saxton and Sabatini note:

Early studies into the biological properties of rapamycin revealed a role in blocking lymphocyte proliferation, leading to its eventual clinical approval as an immunosuppressant for kidney transplants in 1999. The immunosuppressive action of rapamycin is largely attributed to its ability to block T cell activation, a key aspect of the adaptive immune response.

Mechanistically, mTORC1 facilitates the switch toward anabolic metabolism that is required for T cell activation and expansion and lies downstream of several activating signals present in the immune microenvironment, including interleukin-2 (IL-2)*, the costimulatory receptor CD28, as well as amino acids.*

Interestingly, mTORC1 inhibition during antigen presentation results in T cell anergy, whereby cells fail to activate upon subsequent antigen exposure. As the induction of T cell anergy via nutrient depletion or other inhibitory signals is a mechanism utilized by tumors in immune evasion, these data suggest that promoting mTORC1 activation in immune cells may actually be beneficial in some contexts, such as cancer immunotherapy.

Recent studies have also ***found a role for mTORC1 in influencing T cell maturation, as rapamycin promotes the differentiation and expansion of CD4+FoxP3+*** *Regulatory T cells and CD8+ memory T cells while suppressing CD8+ and CD4+ effector T cell populations, consistent with the metabolic profiles of these cell types. Indeed, a recent report found that*

[15] https://www.researchgate.net/publication/338127132_Adaptive_Mutability_and_Cancer

[16] https://www.researchgate.net/publication/336116071_Tumor_Associated_Immune_Cells_On_the_one_hand_and_on_the_other_hand

during the asymmetric division of activated CD8+ T cells, ***mTORC1 activity is high in the ''effector-like'' daughter cell, but low in the ''memory-like'' daughter cell, due to the asymmetric partitioning of amino acid transporters.***

Thus, the role of mTOR signaling in the immune system is clearly more complex than previously thought. Given the current clinical use of mTOR inhibitors in both immunosuppression and cancer, a more comprehensive understanding of how mTOR signaling influences overall immune responses in vivo will be a critical goal going forward

This is a very intriguing observation. We have examined various immunotherapeutic approaches and mTOR and its complexes did not at that time reflect a significant target[17].

8.8 UNINTENDED CONSEQUENCES

As we are learning with targeted immunotherapy there are often many unintended consequences in various therapeutic regimens. CAR-T cells have cytokine storms which in some cases can be deadly. mTOR is a powerful player across all cells and targeting it may not only impact the aggressive cancer cell but a list of many homeostatic cells as well.

[17] https://www.researchgate.net/publication/314090163_Cancer_Immunotherapy_A_Systems_Approach

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