Nanotechnology-Based Targeting of mTOR Signaling in Cancer

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Abstract: Mammalian target of rapamycin (mTOR) is a master regulator of cell growth and metabolism, which is activated in response to intra- and extracellular signals, including nutrients, growth factors, and cellular energy levels. The frequent dysregulation of mTOR signaling in cancer makes it an attractive therapeutic target, and several types of mTOR inhibitors have been developed. Nanoparticle-based mTOR modulators are predicted to target various cancers and deliver as well as release drugs in a controlled manner, resulting in enhanced bioavailability and reduced side effects. This mini-review is focused on the molecular mechanism of nanoparticle-based mTOR modulator action as well as the current development of mTOR inhibitors using nanoparticles. Understanding the biological function of nanoparticle-based mTOR modulators will contribute to the development of efficient nano-therapeutics for the treatment of cancers.

Keywords: mammalian target of rapamycin, cancer, rapalogs, mTOR kinase inhibitor, RapaLinks, nanotechnology, nanoparticles

Introduction

The serine/threonine kinase mammalian target of rapamycin (mTOR) is a master regulator of cell growth that integrates cellular responses to growth factors, nutrient availability, and other diverse environmental signals.¹ Several proteins upstream or downstream of mTOR, as well as mTOR itself, have been reported to be either overexpressed or mutated in a number of cancers.² The hyperactivity of mTOR signaling pathway has been observed to be associated with the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in many human cancers.¹ Indeed, mTOR has been identified as a potential target for the development of molecular therapies to treat cancer.

This mini-review summarizes our current understanding of mTOR regulation, as well as the development of novel mTOR inhibitors. New strategies using nanotechnology to overcome the disadvantages of existing mTOR inhibitors, such as drug resistance, and to enhance the efficacy of current mTOR inhibitor-based therapies will be discussed.

Mammalian Target of Rapamycin mTORC1 and mTORC2

mTOR, a member of the phosphatidylinositol-3-kinase-related protein kinase (PIKK) family, is a serine/threonine kinase and there are two biochemically and functionally distinct complexes, namely, mTOR complex 1 (mTORC1) and mTOR complex 2

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(mTORC2) (Figure 1).^{3,4} mTORC1 consists of mTOR, regulatory-associated protein of mTOR (raptor), mammalian lethal with SEC13 protein 8 (mLST8), DEP domain-containing mTOR interacting protein (DEPTOR), and proline-rich Akt substrate 40 (PRAS40).⁵ mTORC1 controls cell growth, cell proliferation, and metabolic homeostasis through the integration of multiple extracellular and intracellular signals including nutrients, intracellular energy status, oxygen level, and mitogens.⁶ Ribosomal protein S6 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) are the downstream targets of mTORC1, which regulate protein translation through the ribosomal protein S6 and eukaryotic translation initiation factor 4E (eIF4E), respectively.^{7,8} mTORC1 controls the expression and maturation process of the sterol regulatory element-binding protein 1/2 (SREBP1/2) transcription factors, which regulate the expression of fatty acid and cholesterol synthesis-related genes.⁹ mTORC1 also regulates SREBP by controlling the nuclear localization of Lipin-1, a phosphatidic acid phosphatase¹⁰ (Figure 1). Rapamycin

forms a complex with the 12 kDa FK506-binding protein FKBP12 and binds the FRB domain of mTOR in a highly specific manner, leading to the allosteric blockage of mTORC1 through the inhibition of substrate recruitment.¹¹ The tuberous sclerosis 1 (TSC1)/TSC2 complex serves as a molecular hub, integrating upstream signals such as intracellular oxygen levels, growth factors, and energy sensing pathways to regulate mTORC1 activity. TSC1/2 negatively regulates Ras homolog enriched in brain (Rheb), functioning as a GTPase activating protein (GAP)¹² (Figure 1). mTORC2 comprises rapamycin-insensitive companion of mTOR (ric-tor), mLST8, DEPTOR, mammalian stress-activated protein kinase interacting protein (mSIN1), protein observed with rictor-1 (Protor-1), Protor-2, and exchange factor found in platelet, leukemic, and neuronal tissues (XPLN).^{13,14} Even though mTORC2 is activated by growth factors, the regulation of mTORC2 is not fully understood. mTORC2 stimulates Akt, serum and glucocorticoid inducible kinase (SGK), and PKC, thus regulating cell survival, metabolism, and the reorganization of actin cytoskeleton¹⁵ (Figure 1). Despite the

Figure 1 Diagram showing mTORC1 and mTORC2 signaling pathways. Growth factors activate mTOR complex I (mTORC1) through IRS1/PI3K-PDK1-Akt by regulating the tuberous sclerosis complex (TSC)1/2. TSC functions as a GTPase activator protein (GAP) for the small G-protein Rheb, an upstream positive regulator of mTORC1. Amino acids signaling causes mTORC1 translocation to the lysosomes, where Rheb resides, via the Rag GTPases-Ragulator complex. S6K1-rpS6 and 4EBP1-eIF4E are well-known downstream targets of mTORC1 and are responsible for the translation pathway. mTORC1 also regulates lipid synthesis through SREBP and inhibits autophagy by phosphorylating TFEB and ULK1. mTORC2 controls cell metabolism, cell survival, and cytoskeleton rearrangement by activating Akt, SGK, and PKC. Akt activity is regulated by both PDK1 and mTORC2. Dotted lines indicate feedback mechanisms.

lysosomes.^{24,25} The nucleotide states of the RagC/D and RagA/B GTPases are regulated by LeuRS, FLCN, GATOR1, GATOR2, and KICSTOR.^{26–29} LeuRS functions as an amino acid sensor for both RagC/D GTPases and Vps34-PLD1.²⁵ In addition, the following amino acid-specific sensors have been identified: SLC38A9 as lysosomal arginine sensor and Sestrin2 and CASTOR1 as cytosolic leucine and arginine sensors, respectively.^{30–32} Most recently, SAMTOR was recognized as an S-adenosylmethionine sensor in amino acid-induced mTOR signaling.³³

mTOR as a Repressor of Autophagy

mTOR responds to the amino acids from the cytosol, as well as from the lysosome (the products of protein degradation). Absence of amino acids in the cytosol inhibits mTOR signaling and initiates autophagy to increase amino acid levels through protein degradation in the lysosome.³⁴ mTORC1 directly phosphorylates unc-51-like autophagy-activating kinase1 (ULK1) and ATG13, two key early effectors in the initiation of autophagy, inhibiting the induction of autophagy and consequently preventing a futile cycle between mTORC1-stimulated mass accumulation and autophagic degradation (Figure 1).¹ mTORC1 also phosphorylates UVRAG to enhance the association with RUBICON and subsequently decrease the interaction with HOPS complex, a component of late endosomes and lysosomes, blocking autophagosome and endosome maturation.³⁵ In addition, mTORC1 regulates the expression of genes involved in lysosomal biogenesis by controlling nuclear translocation of both transcription factor EB (TFEB) and the related transcription factor E3 (TFE3)^{36,37} (Figure 1). During long-term starvation, mTORC1 regulates ribophagy, a selective autophagy of ribosome, through the autophagy receptor nuclear fragile X mental retardation interacting protein 1 (NUFIP1).³⁸ In this context, ribosome functions as a reservoir of amino acids and nucleotides to maintain cell viability, suggesting that the communication between lysosome and mTORC1 is essential for cell survival in the nutrient-deprived conditions.

The Role of mTOR in Metabolism

The functional regulation of mTOR pathways is more complex at the organismal level. mTOR pathways sense the nutrition and energy status and regulate tissue-specific catabolic or anabolic cascades.¹ mTOR signaling enhances adipogenesis, a process to form adipose tissues, the energy storage site in mammals.⁹ In skeletal muscles, insulin activates mTORC2-Akt signaling to induce glucose uptake

and storage of glucose as glycogen, as well as mTORC1 to incorporate amino acids into the muscle biomass.¹ In the liver, mTORC1 also regulates the production of ketone bodies, the energy storage sources for peripheral tissues during fasting.⁹ In obese rodents, hyperactive mTORC1/S6K1 induces IRS-1 phosphorylation at serine residues and consequent IRS-1 degradation, leading to the impairment of PI3K-Akt signaling. Subsequently, insulin resistance results in reduced glucose uptake in skeletal muscles, increased gluconeogenesis in the liver, enhanced FFA release by the adipocytes, and ectopic fat deposition and lipotoxicity in adipose tissue.⁹ In summary, mTOR activity is tightly associated with metabolic dynamics, while its imbalance results in metabolic dysregulation and the development of metabolic diseases such as obesity, nonalcoholic fatty liver disease, insulin resistance, and type 2 diabetes.

mTOR in Cancer

The Role of mTOR in Cancer

As mTOR maintains a balance between cell growth and cell division, dysregulated mTOR signaling has been reported in many cancers.³⁹ An abnormal increase in PI3K/mTOR signaling is observed in a variety of cell lines and murine xenograft models, and plays a role in tumor growth, angiogenesis, and metastasis.⁴⁰ Furthermore, a constitutively active mTOR mutant has been identified in several human cancer cells,² and the upstream and downstream regulators of mTOR have been found to be altered in many human cancer cases.⁴¹

PI3K catalytic activity is also frequently dysregulated in human cancer.⁴² The PI3K/Akt-dependent activation of mTORC1 can be altered by the loss or inactivation of tumor suppressors, including p53, LKB1, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), and TSC1/2, resulting in the promotion of tumorigenesis through increased mTORC1 signaling.⁴³ LKB1 is a positive regulator of AMP kinase (AMPK), which is activated by low intracellular energy levels and inhibits mTORC1 activation by regulating TSC1/2.⁴⁴ Mutations in LKB1 are linked to Peutz-Jeghers syndrome⁴⁵ and lung cancer,⁴⁶ and lead to constitutive activation of mTORC1 under low intracellular energy conditions. PTEN mutations, including silencing and deletions, have been found in glioblastoma, hepatocellular carcinoma, lung carcinoma, melanoma, endometrial carcinoma, and prostate cancer, where they cause the constitutive activation of mTOR signaling.^{47–49} Mutations in TSC2, a negative regulator of mTORC1, lead to tuberous sclerosis syndrome,^{50,51} which is

related to well-vascularized hamartomas and an increased risk of renal cell carcinoma (RCC).^{52,53} Rheb1 is overexpressed in patients with acute myeloid leukemia (AML) and its expression level is associated with the median survival of patients.⁵⁴ At the same time, mTOR mutations in FAT and kinase domains identified in human kidney cancer increase mTOR kinase activity in a Rheb-independent manner, decrease nutrient dependency, and augment cell size.⁵⁵ Various mutations in the FAT domain of mTOR in RCC decrease the binding of endogenous inhibitors, such as DEPTOR and PRAS40, leading to increased mTORC1 and mTORC2 activities.⁵⁶ Furthermore, amplification of rictor or increase in mTORC2 activity is observed in numerous cancers, including those of breast, stomach, liver, brain, lung, and tongue,^{57–59} whereas mTOR overexpression is rarely seen in human cancers.

Increased levels and/or phosphorylation of mTOR downstream effectors in human malignancies are linked to tumor aggressiveness and poor prognosis.^{60,61} For example, S6K1 is overexpressed in lung and ovarian cancers,⁶² and is amplified in some breast carcinomas as well.⁶³ mTORC1 positively regulates eIF4E, a critical rate-limiting initiation factor of cap-dependent translation. The phosphorylation of 4EBP1 by mTORC1 causes its dissociation from eIF4E, allowing it to initiate the translation of its target mRNAs. The levels of eIF4E are elevated in many human tumors, such as breast, colon, late stage head and neck carcinoma, non-Hodgkin's lymphomas, and chronic and acute myelogenous leukemia.^{64–66} In addition, 4EBP1 phosphorylation has been shown to correlate with chemoresistance in ovarian cancer.⁶⁷ The elevated mTOR activity observed in different cancers emphasizes the importance of targeting mTOR in anticancer therapy.

mTOR as an Effector in the Tumor Microenvironment

Recent cancer immunotherapies targeting immune checkpoint inhibitors have achieved remarkable clinical success.⁶⁸ Different types of immune cells play roles in either the promotion or suppression of tumor progression, indicating the importance of controlling the immune cells in the tumor microenvironment. Immune checkpoints, the physiological immunoinhibitory and regulatory components of the immune system, which include programmed-death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), prevent immune activation to maintain immune homeostasis, enhance self-tolerance, and restrict autoimmunity. Hence, the modulation of the tumor microenvironment by the immune

checkpoint inhibitors may be one of the most promising approaches for treating cancers. mTOR controls multiple T-cell fates and functions in adaptive immune cells,⁶⁹ which are necessary for proper T cell function and immune homeostasis.⁷⁰ In addition, mTOR activation is related to PD-L1 expression in human lung adenocarcinomas and squamous cell carcinomas, which leads to immune escape.⁷¹ Furthermore, treatment with rapamycin decreases PD-L1 expression in PTEN-mutant triple-negative breast cancer cell lines in vitro.⁷² Conversely, co-treatment with an immune checkpoint inhibitor and sirolimus (mTOR inhibitor) maintains anticancer efficacy while improving allograft tolerance in patients with melanoma by decreasing the number of cytotoxic T-cells and increasing the number of eosinophils (eosinophilia) and Treg cells in the peripheral blood, and by increasing the number of IFN- γ ⁺ CD4⁺ T cells and serum IFN- γ levels.⁷³ Therefore, the immune-modulatory function of mTOR needs to be considered in cancer therapy when using immune checkpoint inhibitors.

mTOR Inhibitors as Anticancer Drugs

Rapamycin and Its Analogs as First-Generation mTOR Inhibitors

The field of study of mTOR signaling originated from studies on rapamycin, a macrolide obtained from the bacterium *Streptomyces hygroscopicus*.¹¹ Rapamycin was first identified as an anti-fungal and a potent immunosuppressive agent.⁷⁴ The molecular target of rapamycin (TOR) was determined later in a screen of yeast mutants, which were able to proliferate in the presence of rapamycin.⁷⁵ Subsequently, a mammalian TOR (mTOR) was identified by its affinity to FKBP12, an FK506-binding protein.^{76,77} The complex of rapamycin and FKBP12 binds to the FKBP-rapamycin binding (FRB) domain in the C-terminus of mTOR and allosterically inhibits mTORC1 downstream signaling (Figure 3).⁷⁸ mTORC2 is not inhibited by the rapamycin–FKBP12 complex directly; however, prolonged treatment with rapamycin can prevent the newly-synthesized mTOR from associating with rictor, resulting in inhibition of Akt phosphorylation at Ser473.⁷⁹ At the same time, rapamycin decreases the negative feedback inhibition of PI3K signaling via S6K1, leading to upregulation of survival effector Akt. In addition, the effect of rapamycin on 4EBP1 is limited and is cell type dependent.⁸⁰ High resolution cryo-electron microscopy revealed that the rapamycin–FKBP12 complex reduces access of substrates to the active site of mTOR, indicating

that the FRB domain functions as a gatekeeper to the kinase active site.⁸⁰

Rapamycin inhibits the growth of a wide range of cancers, including rhabdomyosarcoma, neuroblastoma, glioblastoma, small cell lung carcinoma, osteosarcoma, pancreatic cancer, leukemia, B-cell lymphoma, as well as breast and colon cancer-derived cell lines.⁸¹ Several rapamycin derivatives have been developed with similar mechanisms of action, but improved pharmacokinetic and solubility properties compared to those of rapamycin: CCI-779 (Wyeth, Madison, NJ, USA), RAD001 (Novartis, Basel, Switzerland), AP23572 (ARIAD, Cambridge, MA, USA), 32-deoxorapamycin (SAR943), and ABT-578 (Abbott Park, IL, USA). The rapamycin analogs CCI-779 and RAD001 show a prolonged bioactivity compared to rapamycin. These rapamycin analogs have been evaluated in cancer clinical trials and have shown anti-proliferative activity against a wide range of cancers.^{82,83} Phase II trials with RAD001 have reached an objective response rate of 47% for Hodgkin lymphoma, 30% for non-Hodgkin lymphoma, and 12% for breast cancers,^{84–86} whereas phase II/III studies with CCI-779 achieved an objective response rate of 4 to 14% for endometrial cancer and 22% for mantle-cell lymphoma.^{87,88}

These findings indicate that rapamycin and a wide range of its analogs can be considered for anticancer therapy. Although rapamycin and the first-generation rapamycin analogs, temsirolimus and everolimus, have shown modest success in clinical trials, the role of rapamycin in inhibiting mTORC1 activity is questionable as rapamycin does not block all mTORC1 functions⁸⁹ and, in addition, reduces the negative feedback inhibition of PI3K signaling via S6K1, which has been shown to lead to upregulation of the survival effector Akt in many cancer cells and clinical samples,⁹⁰ which raise concerns regarding the attenuation of its therapeutic effect. At the same time, mTORC1 functions as a downstream effector of PI3K/Akt and promotes tumorigenesis,⁹¹ indicating that rapamycin therapy could be an attractive therapy when Akt is hyperactivated.

ATP Analog mTOR Inhibitors as a Second-Generation mTOR Inhibitor

A different class of mTOR inhibitors that block the mTOR catalytic site has been developed to address the failure of rapamycin to control all functions of mTOR. Examples of second generation of mTOR inhibitors include Torin,⁹² PP242, pp30,⁸⁹ Ku-0063794,⁹³ WAY-600, WYE-687, and WYE-354.⁹⁴ These novel mTOR inhibitors are ATP analogs

that compete with ATP for binding to the kinase domain of mTOR, leading to the inhibition of mTOR kinase activity (Figure 3). While rapamycin and its analogs, known as allosteric mTOR inhibitors, inhibit only mTORC1, ATP analog inhibitors block both mTORC1 and mTORC2, inhibiting all target activities of mTOR, including Akt phosphorylation at Ser473, and causing a more effective inhibition of cell proliferation.^{89,92} Additionally, the phosphorylation of 4EBP1 at both rapamycin sensitive Ser65 and Thr70, and rapamycin resistant Thr37/46, is suppressed by ATP analog inhibitors; this suppression is associated with the effective targeting of the initiation of translation. Therefore, ATP analog inhibitors provide many therapeutic advantages and are superior to rapamycin and its analogs. Notably, these inhibitors have a much lower half-maximal inhibitory concentration (IC50) values for mTOR than those for PI3K.⁸⁰

Most cancers can survive in hypoxic and energy-poor environments by strongly enhancing glycolysis. The glycolysis-preferential metabolism of cancer depends partially on the Akt-dependent activation of glucose transporter 1 (GLUT1). As ATP analog inhibitors suppress the PI3K-induced activation of Akt and the Akt-dependent accumulation of GLUT1, they reduce glycolysis in cancers more efficiently compared to rapamycin.⁹⁴ This may explain the improved anticancer effects of ATP analogs in xenograft tumors.⁹⁴ Furthermore, ATP analogs also inhibit lipid biosynthetic processes, thereby regulating Akt-dependent SREBP stabilization.¹³

Dual PI3K-mTOR Inhibitors

Even though the results of preclinical trials investigating ATP analogs are encouraging, it has been revealed that cancers can become resistant to ATP analogs through the mTORC1-driven activation of PI3K- and PDK-induced phosphorylation of Akt at Thr308. Even in the absence of Akt phosphorylation at Ser473, the phosphorylation of Akt at Thr308 activates mild substrate-dependent activity, reducing the efficacy of ATP analogs. The newly developed dual mTOR/PI3K inhibitors are expected to solve these problems.⁴³ These dual inhibitors also suppress PI3K effectors through the mTORC1-induced IRS feedback, potentiating their anticancer effects.⁴³ However, the dual inhibitors are only effective in cells with hyperactive PI3K signaling and not in cells with K-Ras hyperactivation.⁹⁵ Concomitant inhibition of PI3K/mTOR and mitogen-activated protein kinase (MAPK) may be required in Ras-driven tumorigenesis.^{96,97} NVPBEZ235 (Novartis), a dual inhibitor of PI3K/mTOR, is currently being tested in several Phase I/II trials for the treatment of

Figure 3 Diagram showing the mTOR structural domain targets for three generations of mTOR inhibitors. mTOR consists of several structural domains: HEAT repeats, FAT (for FRAP, ATM, TRAP), FRB (FK506 binding protein 12 (FKBP12)–rapamycin binding), kinase, and FATC (for C-terminal FAT) domains. As the name implies, the FRB domain is responsible for the binding of mTOR to FKBP12 and rapamycin. FAT, kinase, and FATC domains are required for maintaining phosphatidylinositol 3-kinase-related kinases (PIKKs) activity. Rapalogs, the first-generation mTOR inhibitors, decrease mTOR activity by interacting with the FRB domain of mTOR. The second-generation mTOR inhibitors competes with ATP for binding to the kinase domain of mTOR. RapaLink, the third-generation mTOR inhibitor, was developed to overcome the limitations of previous mTOR inhibitors. FRB domain mutations (mTORA2034V and mTORF2108L) and a kinase domain mutation (mTORM2327I) contribute to the resistance of mTOR to rapalogs and mTOR kinase inhibitors, respectively.

advanced solid tumors and metastatic breast cancer.^{11,98} The combination of NVPBEZ235 and AZD6244, a MAPK/extracellular signal-regulated kinase (ERK) kinase (MEK) inhibitor, showed a significant tumor regression in the K-Ras transgenic mouse model.⁹⁶

RapaLink as a Third-Generation mTOR Inhibitor

It has been reported that clinically relevant mutations in mTOR enhance the catalytic activity of mTOR and consequently decrease the efficacy of mTOR inhibitors and dual PI3K/mTOR inhibitors in cancer cells.^{2,99} In addition, single amino acid substitution (A2034V and F2108L) in the FRB-FKBP12-rapamycin binding domain confers rapamycin resistance, disrupting the interaction of the FRB domain with the rapamycin–FKBP12 complex.² Moreover, a somatic mutation in the kinase domain at the M2327 position induces resistance to the ATP competitive inhibitor AZD8055, resulting in the hyperactivation of mTOR through an allosteric mechanism.⁹⁹

To overcome drug resistance caused by mutations in the FRB domain or kinase domain, the third generation of mTOR inhibitors, RapaLink, was developed (Figure 3). These inhibitors combine the high affinity for the FRB domain of mTORC1 with the effective kinase inhibition of mTOR kinase inhibitor MLN0128 through a unique juxtaposition of two-drug binding pockets.⁹⁹ In addition, although the mTOR kinase inhibitor MLN0128 is more potent than rapamycin, as it can inhibit 4EBP1 more effectively, it has a short retention time in vivo, and a decreased in vivo activity compared to rapamycin.¹⁰⁰ Additionally, RapaLink has an enhanced efficacy towards 4EBP1 compared to rapamycin and is able to bind to FKBP12, which improves its retention time in vivo.¹⁰⁰ Moreover, the binding of FKBP12 to mTOR is increased in RapaLink treated cells, suggesting that the dual binding of FKBP12 to both RapaLink and mTOR functions to augment the efficacy of RapaLink. Therefore, RapaLink functions effectively in both wild type cells and in cells with resistance to rapalogs or mTOR kinase inhibitors to hinder tumor growth and signaling.

Nanoparticles

Even though mTOR inhibitors have been investigated as potential cancer therapies against a wide range of tumors, their clinical development has been impeded by their pharmacokinetic limitations and the efficiency of their delivery to the target tissues. The increasing interest in nanoparticle-based drug delivery systems is aimed at improving the current cancer therapies, as these systems are able to overcome multiple biological barriers and release their therapeutic load in the optimal dose range.

The Characteristics of Nanoparticles

Nanomedicine is a multifaceted field that employs nanotechnology for diagnosis, therapy, and other medical applications.^{101–104} The definition of term “nanoparticle” is currently expanding to include structures of up to several hundred nanometers, which are formed by the precise assembly of individual components¹⁰⁵. Nanomaterials have a large surface area to volume ratio compared to the larger scale materials, providing them with distinct physicochemical properties. Following the success of lipid-based vesicular drug delivery nanoparticles, many different nanoparticle compositions have been developed, including polymeric micelles, dendrimers, drug conjugates, and polypeptide- and polysaccharide-based nanoparticles. Among them, Genexol-PM (methoxy-PEG-poly (D, L-lactide) Taxol), a polymeric micellar nanoparticle, was the first to be evaluated in phase II clinical trials.

Compared to a single drug, using nanoparticle drug delivery systems offers several advantages: improved efficacy, bioavailability, solubility, and stability of drugs.¹⁰⁶ Nanocarriers have the potential to enhance the effectiveness of chemical entities by optimizing their pharmacokinetic and biochemical properties. Therefore, nanocarriers allow patients to endure higher doses of drugs without experiencing side effects. Recently, nanocarriers have accelerated the development of multifunctional systems for targeted drug delivery and combined therapies and systems for simultaneous therapeutic and diagnostic applications.

The Targeting Efficacy of Nanoparticles

Higher nanoparticle delivery efficiency increases the interaction of nanoparticles with the tumor tissue. In addition to small size and susceptibility to modifications, nanoparticles can passively target solid tumors due to their enhanced permeability and retention (EPR) effect.^{107,108} The abnormalities of tumor vasculature and hypervascularization can be

attributed to changes in vascular architecture and extensive production of vascular permeability factors, which stimulate extravasation within the tumor tissues, lacking lymphatic drainage.¹⁰⁷

Notably, the median efficiency of injected nanoparticles delivery was reported to be only 0.7% across 117 studies between 2006 and 2016, despite recent advances in nanotechnology.^{109,110,111} Most administered nanoparticles do not reach their targeted tissues or organs due to their accumulation and sequestration in the liver and spleen (if >6 nm) or elimination through the kidney (if <6 nm).¹¹² Mononuclear phagocytic system (MPS) in liver and spleen recognize nanoparticles as foreign substances.¹¹³ Therefore, to increase the delivery efficiency, it is necessary to prevent blood clearance of nanoparticles by liver, spleen, or kidney, allowing accumulation in the target tissues.¹¹³

After nanoparticles enter the liver, they transverse through the hepatic sinusoid and then may be sequestered by the Kupffer cells, the tissue resident macrophages in liver sinusoids.¹¹² Kupffer cells are important for the targeting efficiency of nanoparticles; they phagocytose and destroy foreign materials and pathogens in blood, and are involved in erythrocytes recycling. Kupffer cells function as the first layer of innate immunity and the surface charge, ligand chemistry, and size of nanoparticles severely affect the uptake and the retention of nanoparticles.¹¹² Hence, in order to reach to the targeted organ, many applications were developed to block the interaction and the removal of nanoparticles by Kupffer cells (Table 1).¹¹² Once the nanoparticles transcytose through the hepatocytes, nanoparticles may travel through hepatic ducts, accumulate inside the gallbladder, or enter into the common bile duct, and then are excreted into the duodenum of small intestines.¹¹²

However, physiological barriers such as tumor penetration, tumor heterogeneity, relative hypoxia, and endosomal escape may affect EPR effectiveness, necessitating the development of active targeting strategies.¹²⁹ The surface of nanoparticles can be modified with different ligands such as antibodies, aptamers, peptides, or small molecules, which enable them to recognize tumor-specific antigens and thus, actively target cancers and remain at the target site.¹³⁰ Nanoparticle-cell interactions are determined by the protein layer (corona; opsonin) that forms around nanoparticles in the plasma, inducing the subsequent internalization of nanoparticles by defined pathways, such as the endo-lysosomal pathway.¹³¹ The protein corona may mask the targeting ligands, reducing the uptake of nanoparticles in the cell membrane and the targeting capacity of surface functionalized

Table 1 Summary of Applications to Prevent Nanoparticle Uptake by Mononuclear Phagocyte System (MPS)

Counteracting Strategies to Uptake of Nanoparticle by Mononuclear Phagocyte System (MPS)	Kupffer Cell Functionality	Refs
Surface modification	Full function	114–116
Modulus of nanoparticles	Full function	117,118
Saturation of the Kupffer cell phagocytic response	Saturated	119,120
Transient depletion of Kupffer cells	Dead, loss of function	121–124
Using and modeling nanocarrier design from nature	Full function	125–128

nanoparticles.⁴² Moreover, protein corona elicits conformational changes on the surface of nanoparticles, leading to changes in accessibility and subsequent cellular signaling.¹³² The presence of a protein corona on the nanoparticle surface may introduce undesirable interactions between nanoparticles and the immune system, resulting in immunostimulation or immunosuppression.¹³³ Therefore, the new challenges now are to predict protein corona on the surfaces of nanoparticles, or to limit the interactions of nanoparticles with serum proteins and the immune system using smaller antibody fragments (eg, scFv, Fab, F(ab')₂, etc.), or other homing molecules (eg, aptamers, natural ligands, etc), functionalizing nanoparticles with self-markers, respectively.¹³³

Nanoparticles as Regulators of mTOR Signaling

The Effect of Nanoparticles on mTOR Signaling

Cancer cell lysosomes show an increased hydrolase activity during lysosomal biogenesis, leading to the destabilization of lysosomes.¹³⁴ In addition, lysosomes play a critical role in amino acid-induced mTORC1 activation¹³⁵ (Figure 2). Most nanoparticles accumulate in the acidic vesicular organelles, such as endosomes and lysosomes,^{136,137} which contain hydrolytic enzymes that induce the degradation of nanoparticles. Some nanoparticles affect either the stability of lysosomes or autophagy, leading to cell death.¹³⁸ Therefore, targeting lysosomes using nanoparticles may present an effective strategy for anticancer therapy via the regulation of mTOR signaling (Table 2).

Silica nanoparticles (Nano-SiO₂) have been widely used because of biocompatibility, ease of modification, and large surface area.¹⁴³ Nano-SiO₂ specifically localizes in the lysosomes and induces autophagy in endothelial cells, as demonstrated by an increase in autophagy ultrastructures and LC3-I/II conversion.¹⁴⁰ Furthermore, Nano-SiO₂ blocks the PI3K/Akt/mTOR pathway, leading to endothelial cell dysfunction.¹³⁹

Various modifications of nanoparticle surfaces have different effects on the stability of lysosomes. The sequestration of protons by unsaturated amino groups on the surface of nanoparticles can block the V-ATPase proton pumps and retain water in lysosomes. This “proton sponge effect” may lead to the swelling of lysosomes, followed by the leakage of lysosomal contents, lysosome rupture, and finally apoptotic cell death.¹⁴⁴ However, a specific modification with a carboxyl group, such as the modification of polystyrene nanoparticles with a carboxyl group (PS-COOH), does not affect lysosomal stability, whereas modification of polystyrene nanoparticles with an amino group (PS-NH₂) leads to the elevation of pH levels in the acidic vesicular organelles and an impaired lysosomal function.^{142,145} Two kinds of functionalization of nanoparticles regulate mTOR signaling differently: PS-COOH induces mTOR activation, while PS-NH₂ inhibits mTOR signaling; this indicates that nanoparticle surface modifications are important for the regulation of

Table 2 Modulation of mTOR Signaling by Nanoparticles

Nanoparticles	mTOR Activity and Related Signaling	Biological Effect	Refs
Silica	Activation of mTOR and Akt	Disruption of NO/NOS, induction of inflammatory responses and autophagy induction	139,140
PS-NH ₂	Inhibition of mTOR and Akt, Activation of AMPK	ER stress, ROS generation, autophagic cell death	141
PS-NH ₂	Inhibition of mTOR, S6K1, and Akt	Cell cycle arrest, apoptosis, autophagy induction	142
PS-COOH	Activation of mTOR, S6K1, Akt	Reduction in necrotic cells	142

mTOR activity.¹⁴² However, since the modulation of mTOR action by nanoparticles has low specificity, conjugating selective mTOR inhibitors with nanoparticles could be a promising approach to increase their therapeutic effects.¹⁴⁶

Rapamycin–Nanoparticle Conjugates

Polymer-drug conjugates are one of the most dominant classes of therapeutic products based on nanotechnology due to their higher pharmaceutical efficacy compared to the conjugated drugs.¹⁰³ These conjugates of therapeutic products with nanoparticles have been developed to improve the efficacies of previously approved drugs, rather than designing entirely new ones.^{147,148} A rapalog sirolimus (Rapamune, Pfizer, New York City, NY, USA), only available as an oral formulation, has a low bioavailability, since it is too hydrophobic for the preparation as an injectable formulation.¹⁴⁹ The low bioavailability of sirolimus restricts its usage to low-dosage treatments, such as those used for immunosuppression in renal and liver transplant recipients.¹⁵⁰ Polymeric nanoparticles (PNPs) containing sirolimus have been shown to last in blood and disperse easily in physiological media without the loss of free sirolimus effect against the cancer cells.¹⁴⁷ Furthermore, the PNP-sirolimus combination therapy has an improved radiotherapeutic effect compared to sirolimus only, leading to drastic inhibition of tumor growth.¹⁴⁷

Although nanoparticle conjugation has resolved issues associated with single-rapamycin cancer therapy, its use is still significantly restricted due to low bioavailability and resistance profiles. Therefore, co-delivery of rapamycin with other drugs is an efficient strategy for solving problems related to single-rapamycin therapy and improving the anticancer effects. Nanoparticles such as poly (lactic-co-glycolic acid) (PLGA) nanoparticles serve as carriers encapsulating the active ingredient being used for the targeted treatment of cancers. Recently, Rapa-loaded PLGA nanoparticles (Rapa-PLGA-NPs) coated with P80 (Rapa-PLGA-P80-NPs) showed anti-glioma activity in vitro.¹⁵¹

Co-delivery of rapamycin- and piperine-loaded polymeric nanoparticles improved the oral bioavailability and efficacy of rapamycin and piperine.¹⁵² Piperine (PIP) is a natural alkaloid, well known for the enhanced intestinal permeability as a P-glycoprotein (P-gp) inhibitor, as well as mild anticancer activity.^{153–155} Multidrug resistance (MDR) in tumors is caused by P-gp, an efflux transporter, leading to low drug bioavailability.¹⁵² Using poly (D, L-lactide-co-glycolide) (PLGA) nanoparticles, the co-delivery of

rapamycin and piperine reduced their doses and improved their bioavailability through the inhibition of P-gp efflux and increased uptake by the breast cancer tissues.¹⁵²

The use of cisplatin is restricted by systemic toxicities coupled with drug resistance, even though cisplatin has been widely used against a broad spectrum of solid neoplasms.¹⁵⁶ Encapsulation of cisplatin together with rapamycin inside PLGA nanoparticles led to the synergistic effect of the two drugs through the alteration of the tumor microenvironment.¹⁵⁷ In many solid tumors, an increased interstitial fluid pressure (IFP) builds a barrier to trans capillary transport, resulting in inefficient uptake of therapeutic agents.¹⁵⁸ Therefore, remodeling the tumor microenvironment might be an effective strategy for the sensitization of tumor cells to drug treatment.¹⁵⁸

Concluding Remarks and Future Directions

In the last decade, considerable progress has been made in understanding mTOR signaling and the potential of mTOR-targeted anticancer therapies; however, significant aspects of mTOR regulation need to be further clarified. The growth inhibition characteristics of rapamycin and its analogs stimulated investigations of their anticancer properties. Despite several promising findings in preclinical studies, investigations of rapalogs in cell lines and patients have shown a limited clinical impact, including selective inhibition of mTORC1 and activation of feedback loops. One of the impediments to progress in mTOR inhibition therapy is the lack of predictive biomarkers to determine its effectiveness and lack of information regarding the mechanisms of cancer unresponsiveness to this therapy. Additionally, the direct mTOR kinase inhibitors, ATP analogs, are likely to have off-target effects on other related kinases, causing an increase in toxicity. These limitations in the use of current mTOR kinase inhibitors prompted the improvement of the efficacy of these drugs using nanoparticles.

The development of nanocarriers containing mTOR inhibitors aims to optimize drug delivery to the target tissues. Although rapamycin–nanoparticle conjugates showed improved bioavailability compared to sirolimus alone (see 6.2), the molecular mechanisms of nanoparticle-induced modulation of mTOR signaling need to be investigated to maintain the selective therapeutic effect on cancers. Also, the interplay between the lysosome-mTORC1 and nanoparticles in cancer cells warrants further investigation, since both mTORC1 activation and nanoparticle

accumulation occur at the lysosome. Further development of nanocarriers for the second and third generations of mTOR inhibitors will enable the emergence of cancer therapeutics targeting mTOR with high efficacy. Therefore, it is of high importance to investigate a combination of selective nanoparticles and mTOR inhibitors to treat specific cancers.

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Disclosure

The author reports no conflicts of interest in this work.

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