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Targeting the PI3K/Akt/mTOR Signaling Pathway: Applications of Nanotechnology

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Abstract

Mammalian target of rapamycin (mTOR), as an axial mediator of multiple cell growth pathways, is in connection with several other proteins that are involved in the regulation of homeostasis in the cell function. mTOR's signaling pathway participates in and integrates a variety of environmental cues to control cancer cell and normal tissue development. mTOR and its inhibitors including the rapamycin analogues are attractive therapeutic indication to clinical trials for treating various types of cancers, with or without inhibitors of other signaling pathways. Despite the promising results in cancer treatment, low water solubility of rapamycin is shown to decrease its therapeutic efficacy. To reach an acceptable level of efficacy, high distribution and accepted dispersing of utilized drugs in control of mTOR signaling pathway, nanomaterials-based drug delivery can play an important role. Evaluation of the mechanisms and therapeutic effects of nanoparticle-based mTOR modulation can be useful in developing safe strategies in treatment of cancer. Regarding the clinical importance of mTOR deregulation in human diseases, hereby, we address the recent progress in the field of nanoparticle-based mTOR targeted therapy.

Keywords: mTOR, Molecular targeting, Nanotechnology, Cancer

Introduction

Mammalian target of rapamycin (mTOR), as a central mediator of multiple cell growth pathways, is in connection with several other proteins that are involved in the regulation of cell growth. This system comprises of mTOR complex 1 and mTOR complex 2 that control different cellular processes through phosphorylation of key translation regulators such as ribosomal S6 kinase and eukaryote initiation factor 4E binding protein. Mechanistically, mTOR is a central sensor for physiological responses such as the levels of oxygen, nutrient and energy of the cell (1,2). Therefore, mTOR is considered as a molecular target, because of involving in the several cellular processes, and several studies have shown its implication in the various human diseases. In other words, deregulation of mTOR has been reported in cancer, obesity and depression. The advent of these diseases has been led to the development of rapamycin, as the best known inhibitor of mTOR, and its analogs (3). Mechanistically, rapamycin and its analogues inhibit mTOR by binding to its non-catalytic domain. Due to their extreme selectivity, these chemicals are used in the clinic to specifically target mTOR (4). Nevertheless, they need to be distributed homogenously inside the target tissues and cells in order to reduce their possible systemic side-effects. One of the established strategies to achieve controlled delivery of drugs is to use nanoparticulate systems. Nanoparticles of various characteristics are available for optimal drug delivery e.g. for increasing their stability in the blood circulation and targeted delivery to the tumors by taking the advantage of enhanced permeability and retention effect near the tumor tissue (5,6). These nanoparticles are composed of different materials such as magnetic, inorganic and organic-based components. Therefore, different types of nanoparticles have been used to inhibit mTOR activity in both normal and cancer cells, leading to the dramatic decrease in the level of phosphorylated mTOR and subsequently mTORC1 catalytic activity (7). Regarding the clinical importance of mTOR deregulation in human diseases, hereby, we address the recent progress in the field of nanoparticle-based mTOR targeted therapy (8).

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Biology of mTOR

mTOR, a 300 kDa serine/threonine protein kinase and a well-known member of phosphatidylinositol kinase related kinase (PIKK) family, functions as a molecular sensor of cellular starvation and its subsequent cellular event, autophagy (9). mTOR is involved in a pathway, known as PI3K/Akt/mTOR signaling pathway that regulates cellular growth behaviors which has the potential to lead to tumorigenesis. Recently, it has been revealed that mTOR does its function by mediating the signal between two distinct complexes knowns as mTOR Complex1 (mTORC1) and mTOR Complex2 (mTORC2). mTORC1 receives the signal from AKT, while mTORC2 controls AKT activity through Ser473 phosphorylation (2). To be activated, AKT needs to be phosphorylated at Thr308 by phosphoinositide-dependent kinase-1 (PDK1).

For activation of PI3K/Akt/mTOR pathway, growth factors trigger the signaling by binding to their receptors and subsequent activation of receptor substrates. Then, phosphoinositide 3-kinase (PI3K) binds to the intracellular part of the activated receptor and converts phosphatidylinositiol-4, 5- phosphate (PIP2) to phosphatidylinositiol-3, 4, 5-phosphate (PIP3). PIP3, in turn, activates PDK1 to AKT route that results in the activation of mTOR. As a tumor suppressor protein, phosphatase and tensin homologue deleted on chromosome 10 (*PTEN*) can indirectly inhibit mTOR activation by reversing the PIP2-to-PIP3 reaction and subsequent AKT inhibition (1). From the molecular point of view, it has been revealed that rapamycin can interfere with the function of raptor–mTOR complex through inhibition of cell growth and the subsequent diminish in the cell size and, as a raptor is involved in several molecular processes including translation, metabolism and autophagy (10). mTORC1 activity is regulated by different pathways, particularly through the growth factor/PI3K/Akt pathway (4). On the other hand, Akt has a dual role in the signaling of mTOR as it activates mTORC1 and is activated by mTORC2. Tuberous sclerosis complex (TSC) 2 is the mediator of Akt for modulation of mTORC1 activity (11). When makes the complex with TSC1, the heterodimer converts Rheb to an inactive GDP-bound state by GTPase-activating protein (GAP) activity existent in the complex that leads to the inhibition of mTOR activity. Nonetheless, growth factors activate Akt, and, it, in turn, inhibits GAP activity through phosphorylation of TSC2 at Thr1462 and Ser939. This leads to an active Rhen form and thus, the active form of mTORC1 which expands the signaling to S6K and 4EBP1 (4,12) (Figure 1).

mTOR Upregulation in Cancer

PTEN gene, as one of the most frequently mutated genes, mutations are the most well-known genetic factors associated with mTOR signaling that results to the emergence of several cancers such as melanoma, lung, breast, prostate, endometrial, bladder, brain, thyroid and renal cancers (13). *PTEN* mutations cause up-regulation of mTOR in cancers that do not have good prognosis, are not well differentiated and have a high recurrence rate, particularly hepatocellular carcinoma (HCC). Nonetheless rapamycin and its analogues, known as rapalogs, can effectively inhibit the growth of HCC in animal models (14).

Various mTOR Inhibitors

To inhibit the activity of mTOR, rapamycin and its analogs attach onto a non-catalytic domain of the molecule in a highly selective manner. This extreme selectivity has led to the clinical application of these compounds to treat cancer (4). Structurally, Rapamycin (sirolimus) belongs to the macrolide family and had been used as an antifungal compound. Later on, it was used as immunosuppressor and anti-cancer agent. Although the exact mechanism of mTOR inhibition has not been fully elucidated, some studies suggest that rapamycin acts through binding to its intracellular receptor namely FKBP12 as a complex and to the mTORC1, suppressing its phosphorylation on S6K1 and 4EBP1 (15). Currently, AP23573, CCI-779 (temsirolimus) and RAD001 (everolimus) are used as analogs of rapamycin in the clinic. They are being used for treatment of breast, non-small-cell lung and renal-cell cancers. Also, they are well tolerated in skin reactions, mucositis and myelosuppression (13). The specific nature of these analogs originates from their selective interaction with FKBP12 and mTOR, but not other proteins (16).

Beside the rapamycin and its analogs as specific inhibitors, ATP analogs are used as a novel generation of mTOR inhibitors that suppress the kinase activity of mTOR by competition with ATP molecules, as physiological phosphate donor, for binding onto kinase domain. Therefore, regarding the mechanism of action, ATP analogs can inhibit both mTORC1 and mTORC2.

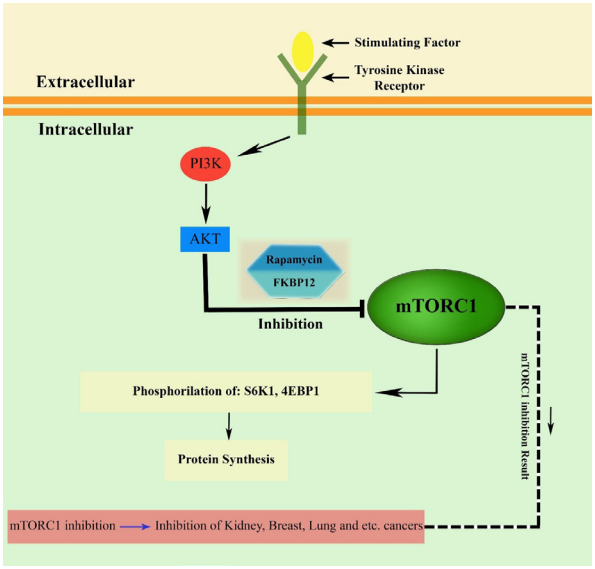

Figure 1. mTOR Signaling Pathway and its Inhibitors.

Table 1. mTOR Inhibitors

Type of mTOR Inhibitors	Mechanism of Action
Rapamycin and analogues	Binding to the immunophilin FKBP12 Partial mTORC1 inhibitor
Inhibitors of kinases (ATP analogues)	ATP competitive inhibitor pf mTOR, inhibition of mTORC1 and mTORC2
PI3K inhibitors	ATP competitive inhibitor of PIK3 and mTOR

Moreover due to the presence of a similar kinase domain between mTOR and the PI3Ks, some of the ATP analogs are capable of inhibiting PI3K. On the other hand, some PI3K inhibitors such as LY294002, wortmannin, theophylline65 and caffeine have been shown to have mTOR-inhibitory activities (17). We summarized some inhibitors of mTOR in Table 1. In addition to anti-cancer properties, mTOR inhibitors have been shown useful in minimizing vascular intervention-induced restenosis by inhibition of vascular smooth muscle cells (VSMCs) growth. However, regarding the anti-restenosis effect of mTOR inhibitors, no direct link between this disease and mTOR activity has been found (18). As another drug that indirectly perturbs mTOR activity, perifosine has been found to inhibit protein kinase B/Akt phosphorylation in PC-3 prostate carcinoma cells, showing anti-growth property on these cells; and currently, perifosine has shown promising results in phase 1 clinical trials (19).

Immunosupressive effects of mTOR inhibition

In addition to its anti-cancer properties, rapamycin is known as a strong immunosupressive agent which originates from its ability in disturbing the signaling pathways of cytokines involved in the induction of proliferation and differentiation of lymphocytes. As an instance, it keeps the IL-2 induced T cells in G1 phase and guides them into a mid-to-late Gl arrest (20). Furthermore, as an agonist, cyclosporine A synergizes the immunosuppressive effect of rapamycin as shown in reducing the rejection rate in renal transplantation (16).

Side Effects of mTOR Inhibition

Despite the promising results in cancer treatment, rapamycin is shown to decrease hepatic LDL receptor (*LDL-R*) expression as well as the expression of Scavenger receptor, class B, type I (SR-BI) in human umbilical vein endothelial cells (HUVECs) and therefore, it may have side-effects in patients with hyperlipidemia. From the molecular point of view, the main reason for this side-effect is reduction of eNOS that leads to endothelial cell dysfunction and atherogenesis (21).

Nanoparticles

Targeted delivery of therapeutic agents into the disease site is a great challenge in treatment of various human diseases, particularly cancer, because routine drug delivery regimens face with many problems including low efficiency, non-specific targeting and rapid clearance from the body. To address these issues, drug is usually delivered by conjugating them into specifically-designed vehicles. As one of the promising vehicles, nanotechnology-based materials, i.e. nanoparticles as structures smaller than 100 nm in at least one dimension, has been found widespread applications in the targeted drug delivery (22). Nanoparticles solve the mentioned problems through increasing the stability and maintaining the sustained release of the drug, and, thus reducing their side-effects by decreasing the needed therapeutic doses delivered (23,24).

Types of Nanoparticles in Drug Delivery

There exist various types of nanoparticles regarding their composition and original source. Natural-based nanoparticles are often synthesized from the biomolecules, particularly chitosan, lactic acid, dextran, lipids and phospholipids; While chemical-based nanoparticles are frequently made from the synthetic materials such as metals, silica, various polymers and carbon. The cellular systems respond differently into the nanoparticles of different origin; therefore, the nanoparticles should be engineered properly to minimize their adverse effects on the cells, while maximizing the effectiveness of drug delivery (25,26).

Nanoparticles Application in mTOR Inhibition

Similar to other drugs, target cells develop drug resistance against the mTOR inhibitors and cause them to be less-efficient when used in the pure form; probably because the target cells become adapted to the drug by recruiting alternative signaling pathways that have cross-talk with mTOR signaling (27). In order to avoid the drug resistance; various nanoparticles-based systems have been devised to deliver the mTOR inhibitors.

Liposomes

Liposomes which are the bilayer spherical nano/microparticles of 80-300 nm size, have been used as the first vehicles of drug delivery and can be formed spontaneously in aqueous media by mixing a defined ratio of phospholipids and steroids (22). They are frequently used to deliver the hydrophilic and hydrophobic drugs, and are classified into 2 generations of simple ones, i.e. first generation, and the stealth one, i.e. long-lasting type. The main purposes of using liposome-based drug delivery are increasing the pharmacokinetic stabilities in the circulation and the near target tissues; and also minimizing toxicity and immunogenicity of the drug (28).

The main reasons for formulating rapamycin inside liposomes are its high hydrophobicity, poor bioavailability, its susceptibility to the degradation and clearance by erythrocytes. Loading into the lipid bilayers increases the water solubility of rapamycin as lipid bilayer of liposome is hydrophobic and thus retains the drug in the liposome structure by solving it.

Loading of rapamycin into pegylated and conventional liposomes has not been changed by alteration of cholesterol to phosphatidylcholine ratios. It seems the stability of pegylated liposomes was higher in comparison with conventional liposomes. Anti-proliferation characteristic of conventional liposomes against the breast cancer cell line was higher compared to pegylated liposomes. Indeed, conventional liposomes are more suitable in direct administration into tumor such as breast cancer, but in intravenous injection pegylated liposomes are the candidate, in term of permeability and stability (29).

Rapamycin liposome formulations, as efficient alternative compared to the free drug composition, can be used for therapy of breast cancer and they have notable effects on suppressing of metastasis and tumor growth (30). The advantages of rapamycin liposome formulations include stability, fluidity, proper drug distribution/ incorporation and loading the rapamycin into the lipid bilayer (28).

The combinatorial therapy by rapamycin and doxorubicin (DOX) - loaded cyclic octapeptide liposomes is a novel strategy that affected integrin α3 and fought the triple-negative breast cancer (TNBC).

Rapamicin solubilization was increased by loading of PEGePCL polymer micelles (M-RAPA). The studies suggest that to improve therapeutic efficacy of TNBC, the simultaneous administration of LXY-LS-DOX and M-RAPA systems may provide a rational strategy (27).

In a study, the liposomes that had been prepared by the remote film loading protocol, were assessed for the treatment of restenosis by the internal delivery of rapamycin entrapped nanoliposomes. The finding showed, beneficial strategy in restenosis treatment after angioplasty (31).

Dendrimers

Morphologically dendrimer shapes supposed globolar with highly branched tree-like macromolecules with many arms fans out from a central core. One of the most broadly used dendrimer scaffolds in biology is polyamidoamine (PAMAM) dendrimer. In spite of their general usage, to avoid the toxicity and liver damages related with their polycationic surfaces, it is necessary to neutralize the amine groups of these dendrimers with neutral or anionic moieties (32). By deregulating the mTOR and its downstream signaling pathway, PAMAM triggers autophagic cell death. The inhibition of the Akt/ mTOR and activation of the Erk 1/2 signaling pathways were involved in autophagy-induced by PAMAM dendrimers. The autophagy inhibitor 3-methyladenine revealed PAMAM-induced cell death and improved acute lung injury caused by PAMAM (33,34).

Polymeric Micelles

Polymeric micelles could not diffuse to normal tissues and, thus, never recognized by the immune system and this can elongate their circulation lifetime. It also provides passive targeting to tissues that suffer from cancer or inflammation through the enhanced permeability and preservation effect.

Immunomicelles, a class of surface-modified micelles, possess specific ligands for receptors of tumor cell or monoclonal antibodies specific for upregulated antigens on the cancerous cell. The main application of immunomicelles is active tumor-targeting. Stimuli-responsive micelles, are different tools for active tumor targeting that release their drug just in response to physical or environmental stimuli, such as the lower pH in tumor tissue, light, sound, or heat. In a study a stimulus-responsive micelle of poly(ethylene glycol)-b-poly(ε-caprolactone)) loaded with rapamycin has been designed and in vitro release from this micelle has been studied. The results showed that these environmental stimuli such as serum albumin and vitamin E increase the release rate of loaded rapamycin and the results indicate the potential applicability of this micelle in the controlled-release of rapamycin in in vivo (35).

Protein Nanoparticles

In drug delivery for cancer treatment, protein nanoparticle has been used widely. Abraxane (albumin-bound paclitaxel) as protein nanoparticle in combination with rapamycin was used to cure malignant breast cancer. Rapamycin as an immunosuppressive and anti-cancer agent has low water solubility. In order to this solve this problem, albumin is considered as a suitable delivery tool and its efficiency has already been approved in clinic for the treatment of non-hematologic cancers (36).

Similar to other cancer cells, inhibition of mTOR signaling by rapamycin could result in over-expression of Akt phosphorylation. A study has shown that combinatorial therapy with albumin-bound–rapamycin nanoparticles and perifosine can effectively increase the lifespan of animal xenograft model of multiple myeloma (37).

Silica Nanoparticles

The PI3K/Akt/mTOR signaling pathway could be suppressed by Nano-SiO_2. Mechanistically, nano-SiO_2 deregulates the NO/NOS system, induces inflammatory response and it activates autophagy. Moreover, Nano-SiO2 causes endothelial dysfunction through suppressing the PI3K/Akt/mTOR pathway. Based on these results, administration of Nano-SiO2 is a probable risk factor in vascular disorders (38).

Amino-Functionalized Nanoparticles

Inhibition of cell growth and proliferation by various alternatives in cancerous cell lines is a therapeutic goal in clinic. Amine-Functionalized polystyrene nanoparticles (PS-NH2), pause G2 step of cell-cycle, inhibits proliferation and vascularization in leukemia cell lines through the inhibition of mTOR signaling pathways. By contrast, carboxyl groups (PS-COOH) in polystyrene, activate mTOR pathway. Blocking of mTOR affects Akt and p70 ribosomal S6 kinase 1, and other cell cycle regulatory molecules activation. According to the occurrence of autophagy in leukemia cells by both mentioned particles, PS-NH2 increases the permeability of organelles in cells, resulting in apoptosis. On the other hand, primary macrophage show resistance to activation of mTOR by PS-NH2 without any significant cytotoxicity. In brief, the above results indicate the usefulness of functionalized nanoparticles in inhibiting the proliferation of cancer cells without affecting the function of normal cells (39).

Polymeric nanoparticles

Polymeric nanoparticles (PNPs) possess the size of between 10 to 100 nm. Synthetic polymers and natural polymers are the main types of the PNPs. Poly-caprolactone (PCL), polyacrylate, and polyacrylamide are defined as synthetic while albumin, DNA and chitosan gelatin are known as natural polymers. Behavior of nanoparticles in vivo, can be used to classify PNPs as biodegradable, such as polyglycolide (PGA) and poly(L-lactide) (PLA), and non-biodegradable, like polyurethane (22).

Occasionally, PNPs are used as inductive co-factor accompanied by other small molecules to differentiate stem cells. In one study, results showed that PCL/collagen electrospun fibrous scaffold with a small molecule called Ly294002 as a neurogenic inductive in medium, facilitate the differentiation of on the human endometrial stem cells (hEnSCs) into motor neuron-like cells. Attachment of neural-like cells as an important factor improved here in comparison with control groups. These combinations of PNPs looks more suitable for neural tissue engineering and it seems with inhibition of the PI3K/Akt pathway in hEnSCs, differentiation rate has been increased (40). Combinatorial nanoparticles or Chitosan (CS)/ poly (L-lactide) (PLA)/tripolyphotspate (TPP) improved the entrapment efficiency for rapamycin and used to increase its efficacy. Nano-sized microcapsule such as CS/PLA/ TPP average diameter range from 100 to 300 nm. Its homogeneous size distribution, accepted spreading and the entrapment efficiency was increased with the increase of PLA (41).

Carbon Nanomaterials

Carbon nanomaterials, particularly carbon nanotubes (CNT) as one of the most efficient vehicles of drug delivery, are versatile class of nanomaterials that can be readily functionalized with various molecules ranging from biomolecules to chemical drugs. Once functionalized, CNTs can be used for the safe delivery of cargos into the target cells or organs due to their relatively acceptable level of biocompatibility and no immunogenicity, being considered as promising tools in nanomedicine field (42).

This level of relative, but not perfect, biocompatibility has been elucidated through study of the effect of differently functionalized single-walled carbon nanotubes (f-SWCNTs) in vitro and in vivo. In this study, human lung adenocarcinoma A549 cell line was treated with polyethylene glycol (PEG)-, polyaminobenzene sulfonic acid (PABS) - and carboxylic acid (COOH)-functionalized SWCNTs. From three SWCNTs, the COOH-functionalized CNTs exerted a dramatic autophagic effect on the cells through modulating the AKT–TSC2–mTOR pathway and although in vivo study showed an acute lung injury in mice model upon exposure to COOH-functionalized CNTs, the injury could be reversed by blocking the AKT–TSC2–mTOR pathway, thus, blocking the autophagy. This result suggests the inhibition of the AKT–TSC2–mTOR pathway as an effective therapeutic modality for SWCNTs-induced acute lung injury (43).

Magnetic nanoparticles

Magnetic nanoparticles, as nano-tracers, have been used to enhance the resolution of in vivo imaging strategies. In a study, magnetic nanoparticles were applied as contrast agent to increase the resolution of MRI and this dynamic contrast enhanced magnetic resonance imaging has been successfully used to track the changes in angiogenesis upon administration of mTOR inhibitors through imaging the histological changes in the vessels such as possible vascular leaks (44).

Zinc oxide nanoparticles

Zin oxide (ZnO) nanoparticles, metallic nanoparticles of oxide type, have been shown to display photo-induced catalytic and oxidizing effects on various chemical and biochemical molecules (45).

Their pro-oxidant characteristics, particularly against biomolecules, originates from their catalytic properties that emerge as increased levels of free radicals, particularly ROS, resulted from down-regulation of anti-oxidant enzymes by the inhibition of Nrf2 transcription factor release; and induction of lipid peroxidation and protein carbonylation. Besides the induction of oxidative stress, it has been confirmed that ZnO nanoparticles can guide macrophages into autophagy by inducing apoptosis, and the main signaling pathway involved is PI3K/Akt/mTOR pathway in which Akt undergo de-phosphorylation, leading to mTOR inhibition (46).

Hydrogel

As it is obvious from the designation, hydrogels are a class of water-soluble three-dimensional, mesh-like polymers that can absorb water and trap water-soluble molecules

while absorbing water. Being chemically synthesized from a wide range of monomers, they can form different physical structures accordingly and hold promise for controlled released delivery of drug payloads (47). In addition to being used for drug delivery, SF16 peptide-modified hydrogels have successfully been applied in three-dimensional cell culture and tissue regeneration purposes due to offering many beneficial properties, especially inducing growth of the PC12 cells by modulating growth signaling pathways. Those hydrogels increase growth rate of the cultured cells by providing multiple adhesion points for the cultured cells which in turn induce up-regulation of p70S6K1, 4EBP1 and cadherin family, all are regulated by mTOR as a key molecule involved in cell growth. These studies provide an insight into the exact mechanisms involved in the interplay between the adhesion of cells onto the culture matrix and cellular behaviors (48).

Conclusion

Regarding dramatic advances in sciences, cancer still makes a huge part of mortality. Nanoscience accompanied molecular knowledge may be helpful in the treatment of malignancies. Here we analyzed several studies related to the application of nanoparticles in control of the PI3K/Akt/mTOR signaling pathway. Our literature review showed that utilization of nanomaterials in drug delivery system for modulating mTOR can increase the efficacy of cancer treatment while it decreases the unavoidable side effects of drugs.

Competing Interests

Authors declare that they have no conflict of interests.

Ethical Issues

Not applicable.

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