PI3K/AKT/mTOR signalling pathway involvement in renal cell carcinoma pathogenesis (Review)

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Abstract. Renal cell carcinoma (RCC) accounts for over 90% of all renal malignancies, and mainly affects the male population. Obesity and smoking are involved in the pathogenesis of several systemic cancers including RCC. The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signalling pathway regulates cell growth, differentiation, migration, survival, angiogenesis, and metabolism. Growth factors, hormones, cytokine and many extracellular cues activate PI3K/AKT/mTOR. Dysregulation of this molecular pathway is frequently reported in human cancers including RCC and is associated with aggressive development and poor survival rate. mTOR is the master regulator of cell metabolism and growth, and is activated in many pathological processes such as tumour formation, insulin resistance and angiogenesis. mTOR inhibitors are used at present as drug therapy for RCC to inhibit cell proliferation, growth, survival, and the cell cycle. Temsirolimus and everolimus are two mTOR inhibitors that are currently used for the treatment of RCC. Drugs targeting the PI3K/AKT/mTOR signalling pathway may be one of the best therapeutic options for RCC.

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Contents

1. Introduction

In 2012, 143,000 deaths caused by kidney cancer were reported worldwide, with an increased incidence in the Czech Republic, followed by Eastern and Northern Europe, North America and Australia. Africa and South-East Asia had the lowest mortality rates from kidney cancer. RCC accounts for over 90% of all renal malignancies and is characterized histologically by the presence of three cell types: 70% clear cells, between 10 and 15% papillary cells, and only 5% chromophobe cells (1,2). The incidence and mortality of renal cell carcinoma (RCC) in the Czech Republic are among the highest in the world with 27.14 new cases and 11.13 deaths per 100,000 persons per year, while in men, the incidence is 63%, with 40% of cases diagnosed at the advanced or metastatic stage (3).

According to Globocan, in 2018, 403,000 cases of RCC were diagnosed, representing 2.2% of all cancer cases, with 254,000 cases among men and 148,000 among women. In developed countries, the risk of kidney cancer is 0.69% in men and 0.35% in women. In the USA, in 2019, according to published statistics, 74,000 new cases of RCC were reported, representing 4.2% of all cancers (4). Unfortunately, the RCC incidence rate doubled in 2016, being 14.9/100,000 inhabitants, compared to 1975, when the incidence was 7.1/100.00 (4).

In Romania, in 2018, in accordance with results published by Globocan, kidney cancer ranked 12th out of all cancers, representing 2.4%, with a mortality risk of 1.8% and a 5-year survival rate of 2.878% (5). Chronic kidney disease (CKD) and diabetic nephropathy represent other renal pathologies among the adult population worldwide (6-9). The leading cause of death among patients with CKD are cardiovascular diseases, which can be associated with vascular calcification (10-12). Uric acid is a biomarker for the cardiovascular risk, hyperuricemia being associated with endothelial dysfunction, inflammation, and the activation of the renin-angiotensin-aldosterone system (13-15).

Smoking is a risk factor for the development of RCC, the risk increasing by 50% for male smokers, while for female smokers there is an increase of 20%. Obesity is another risk factor for RCC, and a body mass index of 5 kg/m^2 increases the risk by 24% for men and 34% for women (1). Hypertension increases the risk of kidney cancer, affecting the renal glomeruli and tubular apparatus (4,7). In addition, a higher rate of hypertension is reported for CKD patients (16,17). Moreover, male sex, an increased BMI and smoking represent a few risk factors for diabetic nephropathy (18). In the pathogenesis of RCC, in addition to histological characterization, a molecular evaluation is required, which can provide crucial information for future therapeutic targets. The phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) signalling pathway is dysregulated in systemic cancers, including RCC. Hyper-activation of this molecular pathway is correlated with aggressive behaviour of RCC tumours and poor patient prognosis (19,20).

2. PI3K/AKT signalling pathway: Roles, activation, and isoforms

The PI3K/AKT/mTOR, signalling pathway plays a pivotal role in cell survival and growth, being frequently disrupted in malignant pathologies. A frequent enhanced activity of the PI3K/AKT/mTOR pathway in malignant cells is observed, thus the inhibition of mTOR is an attractive strategy to treat cancer (21-29).

Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinases. PI3Ks phosphorylate a component of the eukaryotic cell membrane, namely phosphatidylinositol. Three classes of PI3K (I, II and III) have been identified to date, based on differences in sequence homology and lipid substrate preference (30). Growth factors, cytokines, and hormones bind to receptor tyrosine kinases (RTKs) and G-protein-coupled receptors (GPCRs) and activate PI3K. On the intracellular membrane, class I PI3K phosphorylates the substrate phosphatidylinositol 4,5-bisphosphate (PIP_2) which transforms into phosphatidylinositol 3,4,5-triphosphate (PIP3), recruiting signalling proteins such as AKT (31,32). All activated PI3K classes are involved in a diversity of cellular processes such as proliferation, survival, metabolism, trafficking, and immunity (33). Phosphatase and tensin homologue (PTEN), the main negative regulator of PI3K, dephosphorylates PIP_3 into PIP_2 (34). AKT is activated by two phosphorylation processes. Phosphoinositide-dependent-protein kinase 1 (PDK1) phosphorylates AKT1 at threonine 308, and the second phosphorylation takes place at serine 473 by mTOR complex 2 (35,36). Based on differences in serine/threonine residues, AKT is divided into three isoforms (AKT1, AKT2 and AKT3). AKT1 is ubiquitously present in tissues, being involved in cell growth and survival. AKT2 is present mainly in muscle and adipocytes and contributes to glucose homeostasis, while AKT3 is found in the brain and testes. All three isoforms share more than 80% homology, contain pleckstrin homology (PH), catalytic and regulatory domains and have common specific functions (Fig. 1) (37-41).

3. Structure and function of the mTOR pathway

mTOR is a component of the AKT signalling pathway, which promotes cell growth and proliferation in eukaryotic cells (42,43).

mTOR is a central regulator of cell metabolism, proliferation, growth, and survival. This protein kinase is activated in various pathological cellular processes such as tumour formation, angiogenesis, adipogenesis, insulin resistance and activation of T lymphocytes (42-45). Overexpression of mTOR signalling pathway has been observed in various systemic pathologies such as type 2 diabetes and multiple neoplasms including RCC (42-45). mTOR protein is a serine threonine kinase belonging to the PI3K kinase family, which exists in two distinct multi-protein complexes: mTOR1 and mTOR2 (46).

Both complexes have certain common protein components: mTOR (serine/threonine kinase), mLST8 (lethal mammalian with sec-13 protein 8) and DEPTOR (DEP-domain containing mTOR-interacting protein). The mTORC1 complex additionally contains scaffold protein Raptor (regulatory-associated protein of TOR) and AKT substrate protein PRAS40 (proline-rich AKT substrate 40-kDa) (47,48). Scaffold protein Rictor (rapamycin insensitive companion of mTOR), mSIN1 (stress-activated protein kinase-interacting protein 1) and protein associated with Rictor 1 and 2, named PROTOR1, represent the core components of the mTORC2 complex (Fig. 2) (47,48).

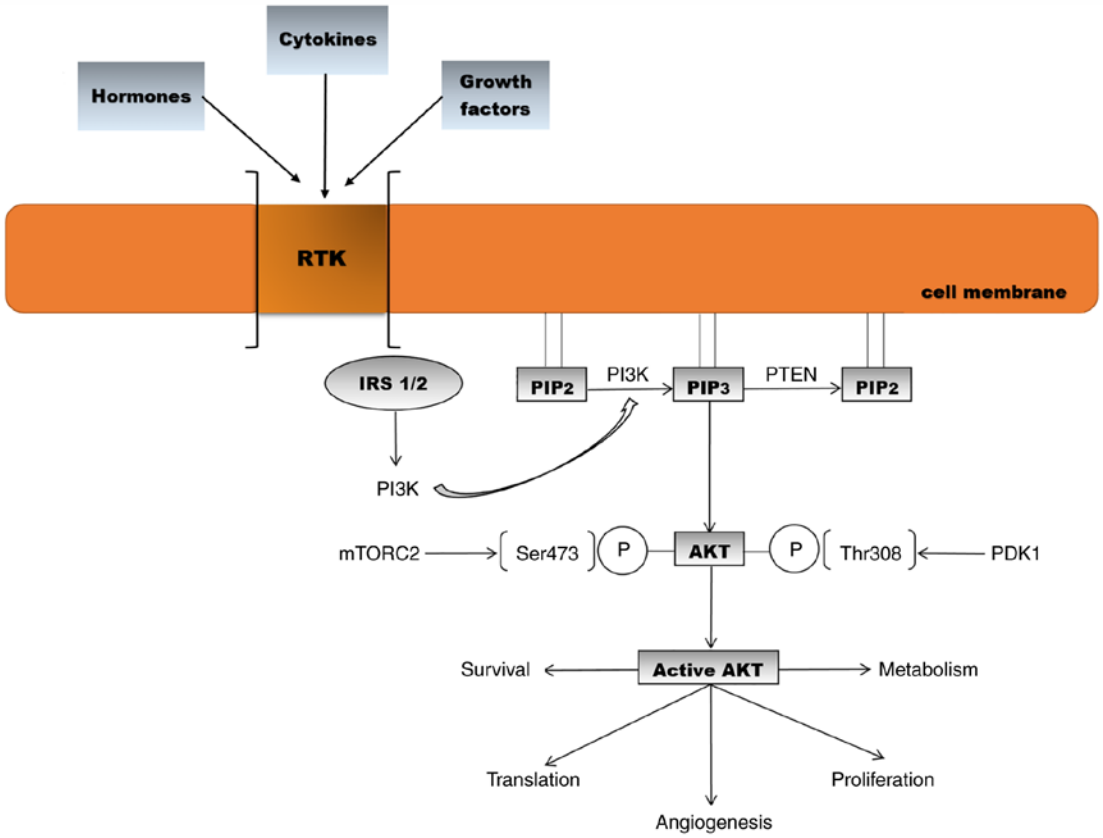

Figure 1. Activation of the AKT signalling pathway and biological effects [adapted from Araki *et al* (2003) and Meric-Bernstam and Gonzalez-Angulo (2009) (32,42)]. RTK, receptor tyrosine kinase; IRS, insulin receptor substrate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-triphosphate; PTEN, phosphatase and tensin homologue; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; PDK1, phosphoinositide-dependent-protein kinase 1; mTORC2. mammalian target of rapamycin complex 2.

In response to environmental factors such as amino acids, stress and growth factors, mTORC1 maintains a cellular balance between catabolism and anabolism. Several growth factors such as insulin, insulin-like growth factor (IGF) and amino acids activate mTORC1 through signalling PI3K/AKT-Tuberous sclerosis complex 1/2 (TSC1/2)-RHEB. PI3K-AKT pathway phosphorylates and inhibits TSC1, which causes RHEB (the small GTPase Ras homologue enrich in brain) activation, which represents the GTPase-activating protein (47,48). Hypoxia and DNA damage, are also signals for mTORC1 activation through TSC1/2 (48,49).

mTORC1 activation promotes protein synthesis by ribosome biogenesis and mRNA translation. Ribosomal S6 kinase (S6K) and the inhibitory Eif4e-binding proteins (4E-BPs) are the major downstream effectors of mTORC1, activated by phosphorylation, which will further phosphorylate other substrata, such as ribosomal protein S6, and protein synthesis initiation factor 4B (eIF4B) (47,50,51). mTORC1 regulates cell growth and proliferation, through its downstream effectors, S6K and 4E-BP, in response to IGF, amino acids, hypoxia and DNA damage (50,52). Overexpression of eIF4E is implicated in malignant transformation of some specific cells (53,54). mTORC1 promotes synthesis of purine nucleotides, *de novo* lipogenesis. Moreover, mTORC1 stimulates glycolysis and glucose uptake through transcription factor hypoxia-inducible factor α (HIFα) (55,56).

mTORC1 is implicated in autophagy-lysosome and ubiquitin-proteasome pathways, involved in protein and organelle turnover. In the nutrient state, ULK1, the mammalian autophagy-initiating kinase is phosphorylated by mTORC1, leading to its inhibition, which further blocks autophagy. Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) activates ULK1 (57,58). mTORC1 activation suppresses the transcription factor EB (TFEB), the most important regulator of the lysosome pathway. mTORC1 inactivation and nutrient deprivation activate TBEB, then its nuclear translocation takes place, leading further to lysosomal and autophagic genes expression (59). Zhao *et al* and Rousseau and Bertolotti report that the ubiquitin proteasome system (UPS) is increased when mTORC1 is inactivated in mammalian cells (60,61). Moreover, mTORC1 controls the translation of cyclin D, c-Myc, and other key proteins involved in cell proliferation (62). Insulin and IGF activate the mTORC2 signalling pathway. The downstream substrata for this kinase protein are less known. Once activated, mTORC2 phosphorylates members of the AGC kinase family, AKT, SGK and PKCα, which are involved in cellular survival, metabolism, and cytoskeletal remodelling. AKT is the most well characterized substratum for mTORC2, being phosphorylated at serine 473 (41,63). Furthermore, AKT phosphorylates TSC2, which is the upstream inhibitor for mTORC1 (41,63). mTOR may be activated by cAMP-dependent protein kinase (AMP kinase), and TSC1/2. In nutrient and energy depletion states, cAMP and TSC 1/2 suppress mTOR (64).

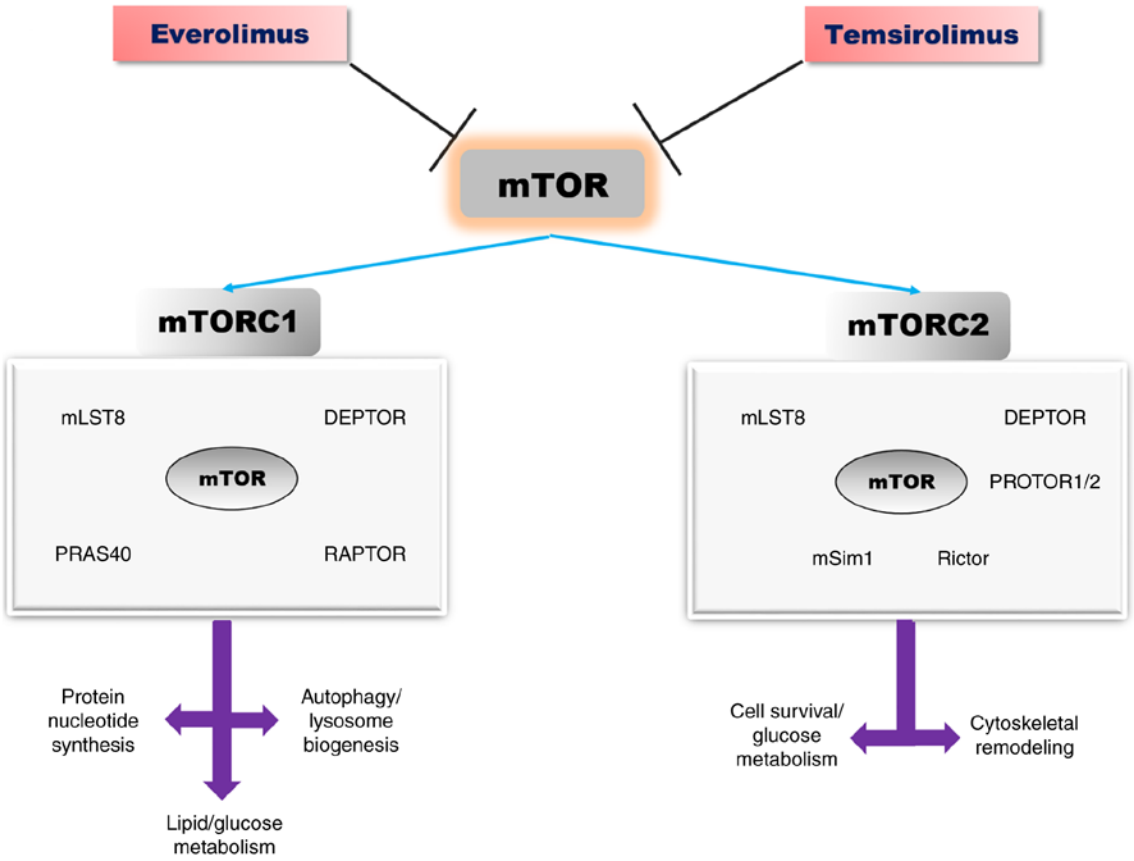

Figure 2. The mTOR signalling pathway: Structure, biological effects, and inhibitors [adapted from Saxton and Sabatini (2017) and Laplante and Sabatin (2012) (47,48)]. mTOR, mammalian target of rapamycin; DEPTOR, DEP-domain containing mTOR-interacting protein; PRAS40, proline-rich AKT substrate 40-kDa; mLST8, lethal mammalian with sec-13 protein 8; Raptor, regulatory-associated protein of TOR; Rictor, rapamycin insensitive companion of mTOR; mSIN1, stress-activated protein kinase-interacting protein 1.

4. PI3K/AKT/mTOR in RCC

In endothelial cells, there are RTKs to which vascular endothelial growth factor (VEGF) binds (VEGFR1/R2), promoting cell proliferation and migration, by activating the mitogen-activated protein kinases (MAPK) and PI3K/AKT/mTOR signalling pathways (65). Receptors for growth factors, such as VEGF, IGF, epidermal growth factor (EGF), have been identified at the level of clear cell RCC (65). Overexpression of EGF, transforming growth factor (TGF)-β, and IGF leads to RTK activation, which further activates various signalling pathways, such as RAS/mitogen-activated protein (MEK)/extracellular signal-regulated kinases (ERK) or PI3K/AKT/mTOR, leading to the production of hypoxia-inducible factor (HIF)-α, which promotes tumour progression (65). Genetic alterations may activate mTOR, reduce the function of PTEN, which cause abnormal activation of AKT, by increasing the function of the catalytic subunit of PI3K (41,65-67). Sato *et al* report mutations in the AKT/mTOR signalling pathway in clear cell RCC (68). In RCC tumours, PI3K/AKT/mTOR signalling pathway activation is correlated with aggressive development and poor survival rate (69,70).

Hyperactivity of mTOR may occur through several mechanisms: Over-activation of growth factors, mutations of the PI3K/AKT signalling pathway, decreased expression of TSC1/2, epigenetic suppression of PTEN and Von Hippel-Lindau (*VHL*) gene inactivation (65,71). Activation of mTOR leads to increased angiogenesis in neighbouring endothelial cells (65,71). In cancer cells, mTOR regulates mRNA translation for HIF1-α, HIF-2α and p70S6 kinase. In the pathogenesis of RCC, overexpression of HIF-1α and HIF-2α, appears to play an important role, together with overexpression of p70S6K (65,72-75). mTOR is involved in upregulation of HIF-α subunits, while VEGF and other molecules increase angiogenesis (65,76). RCC is characterized by alterations of the *VHL* gene. The loss of *VHL* function leads to the deregulation of cyclin D1, a cyclin-dependent kinase cofactor important for cell cycle progression (65,77,78). In clear cell RCC tumorigenesis, the accumulation of HIF-1α and HIF-2α represents a critical step, as a result of bi-allelic alteration of the *VHL* gene. In the presence of phospholipase D, mTOR enhances the expression of HIF-1α and HIF-2α, at the translational level rather than transcriptional (65,79). Chen *et al* identified the activation of the PI3K/AKT/mTOR signalling pathway in the tissues and cells of patients with RCC. The study reports that inhibition of this pathway may reduce epithelial to mesenchymal cell transition in RCC (21). In 2013, Sato and colleagues published the results of integrated molecular analyses in over 100 cases of clear cell RCC, analysing whole-genome and/or whole-exome and RNA sequencing. *VHL* gene defect, HIF accumulation, mutations in the PI3K/AKT/mTOR signalling pathway, p53 and DNA methylation were identified in patients with clear cell RCC genetic lesions (68).

5. mTOR inhibitors in RCC

To date, three methods have been proposed for developing therapeutic agents for RCC: VEGF, RTK and mTOR inhibitors. The mechanism of action of rapamycin drug analogs includes both angiogenesis and tumour cell proliferation. Drug resistance may reduce the utility of mTOR inhibitors (80). Temsirolimus and everolimus are two mTOR inhibitors, which are currently utilized for the treatment of certain RCC subtypes (19,81-84). In May 2007, FDA approved temsirolimus as treatment in a phase III trial, which included 626 patients with poor-prognostic metastatic RCC. The patients received temsirolimus (25 mg intravenous weekly), interferon (IFN)-γ ($3x10^6$ U subcutaneously three-times weekly) or a combination of both (temsirolimus 15 mg weekly and $63x10^6$ U of IFN-γ three-times weekly). The survival rate of patients with metastasis RCC treated with temsirolimus was statistically prolonged when compared with patients who received only IFN-γ (P=0.0069; hazard ratio, HR=0.73). The median overall survival time reported in the temsirolimus group was 10.9 months, 7.3 months in the interferon group and 8.4 months in the combination group. Anaemia, nausea, peripheral oedema, rash, asthenia, hyperlipidemia and hyperglycemia were the common adverse effects observed in patients who received temsirolimus treatment. Based on the results, temsirolimus is the best therapeutic option for metastatic RCC patients. However, the survival rate was not improved in the interferon group by temsirolimus addition (85).

In 2018, Park and colleagues published the results of a phase II clinical trial that included 40 non-clear-cell recurrent or metastatic RCC patients, who received axitinib or temsirolimus. The results of the 3-year study reported promising effects for axitinib in terms of progression-free survival and objective response rate, but not for temsirolimus (86).

In 2017, Bedke *et al* published the results of a study which included patients with metastatic RCC, who received inhibitors for RTK, mTOR and VEGF administered in 3 stages. Axitinib, sorafenib, sunitinib and pozapanib were the RTK inhibitors used in the study, while temsirolimus and everolimus were the inhibitors for mTOR. Sunitinib is the first novel drug, which doubles progression-free survival in patients with metastatic RCC. In the first line of treatment, 626 patients with RCC, divided into three groups-namely, 80% with clear RCC cells, 20% with non-clear RCC and 72% with metastatic RCC, received temsirolimus, INF-α or a combination of the two. Survival rates were higher in patients receiving temsirolimus vs. INF-α (10.9 months compared with 7.4 months). RTK and mTOR inhibitors were administered in the second line therapy, the results being unclear as to the best treatment option. In the third line of treatment, VEGF and mTOR inhibitors (everolimus) were administered, with everolimus showing beneficial effects. Everolimus and temsirolimus reduced S6K1 and 4EBP1 activities and increased the synthesis of HIF1-α. These rapamycin analogues inhibit cell proliferation, growth, and survival, blocking the cell cycle in the G1-phase (84). Until 2006, a high dose of interleukin (IL)-2 remained the best therapeutic target for metastatic clear-cell RCC, but the therapy has evolved and currently three therapeutic targets are proposed. They are inhibitors for RTK-VEGF and mTOR, used in phase III trials, which were found to increase the survival rate (87). In RCC, GNE-477 may be a novel and efficacious dual inhibitor for PI3K-mTOR. Ye *et al* observed, in primary cultured human RCC cells, that GNE-477 inhibited cell growth, viability, proliferation, cell cycle progression, migration, and invasion. GNE-477 inhibited the PI3K-AKT-mTOR signalling pathway in primary RCC cells, by blocking AKT1, p70S6K1, p85 and S6. *In vivo* studies made on nude mice demonstrated that intraperitoneal injection of GNE-477 suppressed tumour growth. GNE-477 inhibited RCC cell growth *in vitro* and *in vivo* (88).

6. Conclusions

Obesity, smoking, and hypertension are risk factors for RCC, representing, in 2018, 2.2% of all cancers worldwide, with a higher incidence in men compared with women. The PI3K/AKT/mTOR signalling pathway is involved in protein and nucleotide synthesis, lipid/glucose metabolism, autophagy, translation, angiogenesis, cell survival and proliferation. Over-activation of the PI3K/AKT/mTOR signalling pathway is crucial for RCC cell survival, proliferation, migration, and metastasis.

Drugs or pharmacological inhibitors of this signalling cascade are promising and important targets for RCC. Temsirolimus and everolimus are two mTOR inhibitors that are used for RCC therapy at present, which have been found to increase the patient survival rate.

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Availability of data and materials

All information provided in this review is documented by relevant references.

Authors' contributions

DM, DGB, AT, OS, IAV, DAM, CCP, RIP, ME, NAS, GV, DEG, AEN and CS designed the review, performed the literature search, selected the included studies and wrote the manuscript and. DM, DGB, AT, OS, IDV, DAM, CCP, RIP, ME, NAS, GV, DEG, AEN and CS critically revised the manuscript. All authors read and approved the final manuscript. The contributions of all the authors on this review are greatly valued and appreciated.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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