

REVIEW

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Resveratrol, curcumin, paclitaxel and miRNAs mediated regulation of PI3K/Akt/mTOR pathway: go four better to treat bladder cancer

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Abstract

Bladder cancer (BC) is a leading cause of death among urothelial malignancies that more commonly affect male population. Poor prognosis and resistance to chemotherapy are the two most important characteristics of this disease. PI3K/Akt/mTOR signaling pathway has been considered pivotal in the regulation of proliferation, migration, invasiveness, and metastasis. Deregulation of PI3K/Akt/mTOR signaling has been found in 40% of bladder cancers. Several microRNAs (miRNAs) have been reported to interact with the PI3K/Akt/mTOR signaling pathway with a different possible role in proliferation and apoptosis in bladder cancer. Thus, miRNAs can be used as potential biomarkers for BC. Natural compounds have been in the spotlight for the past decade due to their effective anti-proliferative capabilities. However, little is known of its possible effects in bladder cancer. The aim of this review is to discuss the interplay between PI3K/Akt/mTOR, miRNAs, and natural compounds and emphasize the importance of miRNAs as biomarkers and resveratrol, curcumin and paclitaxel as a possible therapeutic approach against bladder cancer.

Keywords: mTOR, miRNAs, Biomarkers, Natural therapeutic, Drug resistance

Introduction

Bladder cancer (BC) is among the nine most prevalent types of cancer globally [1]. The male population is mostly affected by this severe disease as BC is among the seventh most common cancers in males. BC accounts for 165,000 deaths annually, and approximately 430,000 cases are reported each year worldwide [2, 3]. In US alone bladder cancer has been responsible for more than 17,240 deaths in the year 2018 and rate of incidence is increasing suggesting that more and more men are suffering from this disease due to occupational exposure to carcinogens

and smoking. Men in the United States are nearly four times more likely to be diagnosed with BC than women and approximately 1 in 27 men in the United States will develop BC in their lifetime. However, in some regions, mortality associated with BC is more frequent in females [2]. The incidence of BC is comparatively less in Asian countries but high mortality rate of BC is reported in Western Asia [4].

BC is a multifaceted disease [5]. The clinical manifestations associated with BC are underpinned by the complex molecular landscape. Cumulative effect of genomic rearrangements, over-expression of oncogenes, loss of tumor suppressor genes and pro-survival signaling pathway integration aggravate this complex anomaly. BC is categorized into non-muscle invasive or muscle invasive carcinoma. Majority of bladder cancers are urothelial carcinomas. Nearly 75% patients are suffering from the non-muscle invasive urothelial

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carcinoma while 25% cases show the symptoms of muscle invasive metastatic urothelial carcinoma [6]. Non-muscle invasive BC is usually low-grade tumor while muscle invasive tumors are high grade tumors. Morphologically, bladder tumors can be divided into papillary, solid, and mixed types. The papillary type is predominant, especially in non-muscle-invasive bladder cancer [6].

Next generation sequencing in combination with in silico approaches have provided in-depth analysis of cancer genome. This has increased our understanding of genomic alterations occurring inside a tumor cell. These approaches have also outlined the drivers behind the deregulated oncogenic pathways and altered biological mechanism responsible for cancer progression. In addition to this the development of potentially more precise treatment for cancer has become more convenient [7, 8]. Despite these advancements finding an effective treatment for BC is still under developed. Resistance to drugs and disease relapse are responsible for high number of deaths in BC [9].

Additionally, deregulated signaling cascades play a crucial role in the progression of different cancers [10]. Cellular aberrations in the phosphoinositide 3-kinase/AKT/mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway have been considered as a promoting factor in the progression of various cancers [11]. The PI3K/AKT/mTOR pathway has been explored extensively to develop therapeutic strategies for different types of cancers [12, 13]. Several mutations in the PI3K/AKT/mTOR pathway cause irregular growth of cancer cells [14].

PI3K and Akt being upstream factors of mTOR might be suitable therapeutic targets in numerous cancers [15, 16]. Development of effective kinase inhibitors that target signaling cascades at different levels has been in focus of scientists for a number of years. However, since PI3K/Akt signaling pathway plays a crucial role in regulating many important cellular events such as cell proliferation, energy homeostasis and survival; adverse effects usually occur due to the toxicity of these inhibitors to healthy cells [17, 18]. In such a situation, the downstream effectors of the PI3K/Akt pathway such as mTOR, can be a target for the treatment of various cancers. Pathway dysregulation affiliated with PI3K/Akt/mTOR has been reported to be detrimental in more than 50% of all human cancers [19]. Up-regulation of mTOR signaling cascade is responsible for tumorigenesis [20]. Mutations of negative regulators of the mTOR such as the Phosphatase and tensin homolog (PTEN), Tuberous Sclerosis protein (TSC1/2) and mTORC1 complex result in an increased severity of disease and decreased responsiveness to drugs. Based on these data it can be concluded

that mTOR can be a potential target for the development of therapeutic strategies for BC.

miRNAs are small molecules (<20–>200 bp) that orchestrate development, differentiation, apoptosis, metastasis and cellular homeostasis [21]. Advancements in high throughput technology, along with microarray and RNA-seq are providing a better insight of the complex role of miRNAs in various cellular pathologies including cancer [22]. Several studies have helped to delineate the involvement of miRNA in regulation of the mTOR signaling pathway. The interplay between miRNAs and mTOR plays a crucial role in mTOR mediated cell proliferation and inhibition. A recent study showed the interplay between *miR-99-5p* and mTOR in benzyl isothiocyanate treated BC cells. There was a reciprocal relationship between miR-99-5p and mTOR expression. Microarray based analysis revealed that there were more than 79 miRNAs dysregulated in benzyl isothiocyanate treated BC cells. Among these dysregulated miRNAs, *miR-99-5p* was up-regulated. *miR-99-5p* expression resulted in the decreased expression of the insulin growth factor 1 receptor (IGF1R) and fibroblast growth factor receptor 3 (FGFR3). This study found also a decreased expression of the mTOR confirmed by the qPCR and western blot suggesting that mTOR was directly regulated by the *miR-99-5p*. These findings indicated that *miR-99-5p* has a role in benzyl isothiocyanate mediated inhibition of cellular growth in the BC cells and miR-99-5p expression helped in cessation of mTOR mediated proliferation in BC [23].

Natural compounds have been extensively used for the treatment of various diseases [24–26]. The anti-cancer efficacy of natural substances has long history. Resveratrol, curcumin and paclitaxel are bioactive molecules with well documented anti-cancer properties. Their interactions with different genes have established them as a modulator of various cancers [27–29]. These compounds help in the regulation of the oxidative stress. Regulation of oxidative stress is an interesting aspect of natural compound mediated anti-cancer activity [27, 30]. Nevertheless, not much has been found about the role of these phytochemicals in the regulation of mTOR mediated signaling. Therefore, this review aims to explain the significant role of mTOR signaling in BC and the interaction between miRNAs and mTOR signaling in BC. The objectives of this review are also to highlight the therapeutic potential of resveratrol, curcumin and paclitaxel and their impact on mTOR signaling in BC.

mTOR signaling pathway

The (Mammalian Target of Rapamycin) mTOR protein is an essential protein kinase which seems to regulate many cellular processes including protein synthesis, cell

growth, and differentiation. mTOR does this under the influence of growth factors, nutrients and stress [31]. There are two complexes—mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) that constitute the mTOR [32]. The mTOR signaling cascade is presented in Fig. 1. Although the expression of these two complexes is strictly maintained in healthy cells, the deregulation of these complexes is a marker for several metabolic diseases and cancers [33]. Normally, mTOR signaling cascade is triggered by the binding of specific growth factor receptors such as the insulin growth factor (IGF), vascular endothelial growth factor (VEGF), platelet derived epidermal growth factor (PDGF) and epidermal growth factor (EGF). The kinases such as the PI3Ks (Phosphoinositide 3 Kinase) have certain regulatory subunits such as the p85 and catalytic subunits p110 that facilitate the catalytic phosphorylation of the (Phosphatidylinositol-biphosphate) PIP2 to PIP3 causing an activation of the signaling cascade [34]. This receptor–ligand interaction promotes the recruitment of the PI3K near receptor site which in turn converts the PIP2 to PIP3 and provides an attachment site for phosphoinositide-dependent kinase 1 (PDK1) [35]. PDK1 attachment to PIP3 promotes the activation of the AKT via phosphorylation. AKT then promotes the activation of several downstream effectors that are involved in the activation of different cellular cascades including mTORC1 causing cellular proliferation. On the other hand, mTORC2 orchestrates activation of AGC family of kinases including AKT, (Serine/threonine protein Kinase 1) SGK1 and (Protein Kinase C) PKC. The targeting of these kinases by various anti-apoptotic drugs inhibits tumor growth and proliferation [36] (Fig. 2).

Metabolism is strictly regulated by the mTOR activation. Any alteration in the signaling cascade causes metabolic changes in glucose, lipids and fatty acids metabolism. Tumor cells are always exposed to stress and therefore they require an additional amount of energy for their survival. Aberrant mTOR signaling provides the cancer cells with abundant amount of energy [12]. Dysregulated glucose metabolism results in increased synthesis of glucose transporter proteins and glycolytic enzyme activation. Glucose transporter 1 (GLUT-1) has been associated with deregulation of the TOR signaling cascade. In most types of cancer GLUT-1 is over-expressed suggesting that GLUT-1 over-expression under the influence of interrupted mTOR signaling cascade results in the activation of oncogenes such as the c-MYC and HIF-1 α [37]. GLUT-1 is member of GLUT family that plays a regulatory role in the homeostasis of glucose by monitoring its intake into the cell's cytoplasm. Glycogen synthase Kinase 3 (GSK-3) negatively regulates the expression of the GLUT-1 gene. Tuberosclerosis complex (TSC) and mTOR positively regulate

the expression of GLUT-1 [38]. mTOR-mediated up-regulation of glucose promotes the process of glycolysis via activation of the hexokinase-2 (HK2) that triggers the phosphorylation of c-MYC and Hypoxia inducing factor 1 alpha (HIF-1 α) [39]. The metabolism of fatty acids is also deregulated by the inhibition of mTOR signaling cascade. mTOR signaling cascade induces the synthesis of fatty acids by activation of the sterol regulatory element binding protein 1c (SREBP) [40].

Modulators of mTOR signaling in bladder cancer

mTOR signaling pathway is necessary for cellular functions for which it needs a huge amount of nutrients and energy. For mTOR pathway to work, AKT is first recruited to plasma membrane where it is phosphorylated at T308 and S473 sites. This in turn results in the activation of AKT. mTORC2 primarily acts as AKT S473 kinase while mTORC1, after being activated by AKT, is essential for cell growth and metabolism modulation. mTOR erroneous signaling is suppressed by its two negative regulators: TSC1 and TSC2 [41]. Mutations in any of these substances cause hyper-activation of mTOR pathway. It has been reported that loss of heterozygosity (LOH) of chromosome 9 occurs in >50% of BC which also has to do with TSC1. Alteration in TSC1 causes mTORC1 signaling up-regulation, leading to tumor formation [42, 43].

Mitogen Activated Protein Kinase-4 (MAPK-4) in different types of cancers is identified as an activating agent for AKT/mTOR signaling via alternative pathway. MAPK-4 activates AKT by phosphorylating it at T308 and it also activates mTORC2 which then fully activates AKT. Activation of AKT promotes cell proliferation and resistance to PI3K inhibitor treatment. MAPK-4 over-expression in BC is directly associated with shorter patients' survival. Knocking down of MAPK-4 induces inhibition of tumor growth and re-sensitization to PI3K inhibitor [44].

In aggressive BCa, transmembrane protein- glycoprotein 130 (GP130) is highly expressed. In an in vivo experiment, its high expression is reported to have a direct association with elevated phosphorylation of mAKT and mTOR, implying it as an obvious cause for rapid tumor growth and aggressiveness. Knocking down of GP130 via siRNA containing nano-particles caused a decrease in cell viability, tumor growth and migration in T24 and UM-UC-3 BC cell lines which suggested that gp130 might be a therapeutic target against tumor growth [45].

Sperm-associated antigen 5 (SPAG5) plays a vital role in mitosis regulation. Its high expression has been reported in both BC tissue and BC cell lines by Liu et al. Its high concentration correlated with higher levels of p-AKT and p-mTOR which suggested that it might have

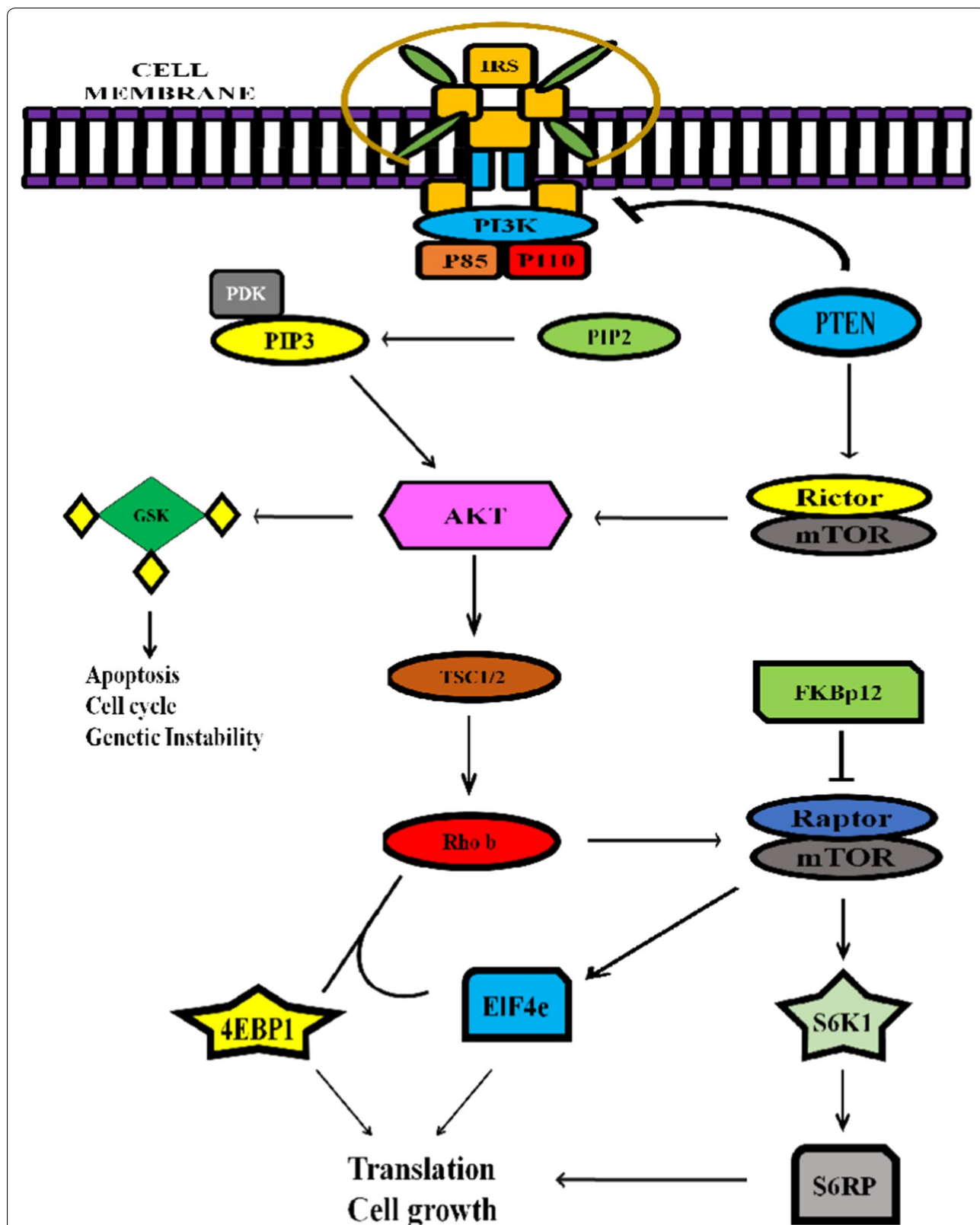


Fig. 1 Schematic representation of mTOR signaling cascade. Classical mTOR signaling cascade is triggered by the binding of ligand to insulin receptor that causes the activation of PI3K and finally initiation of signal transduction from mTORC1 and mTORC2 complexes that allows translation of targeted genes and cytoskeleton rearrangements

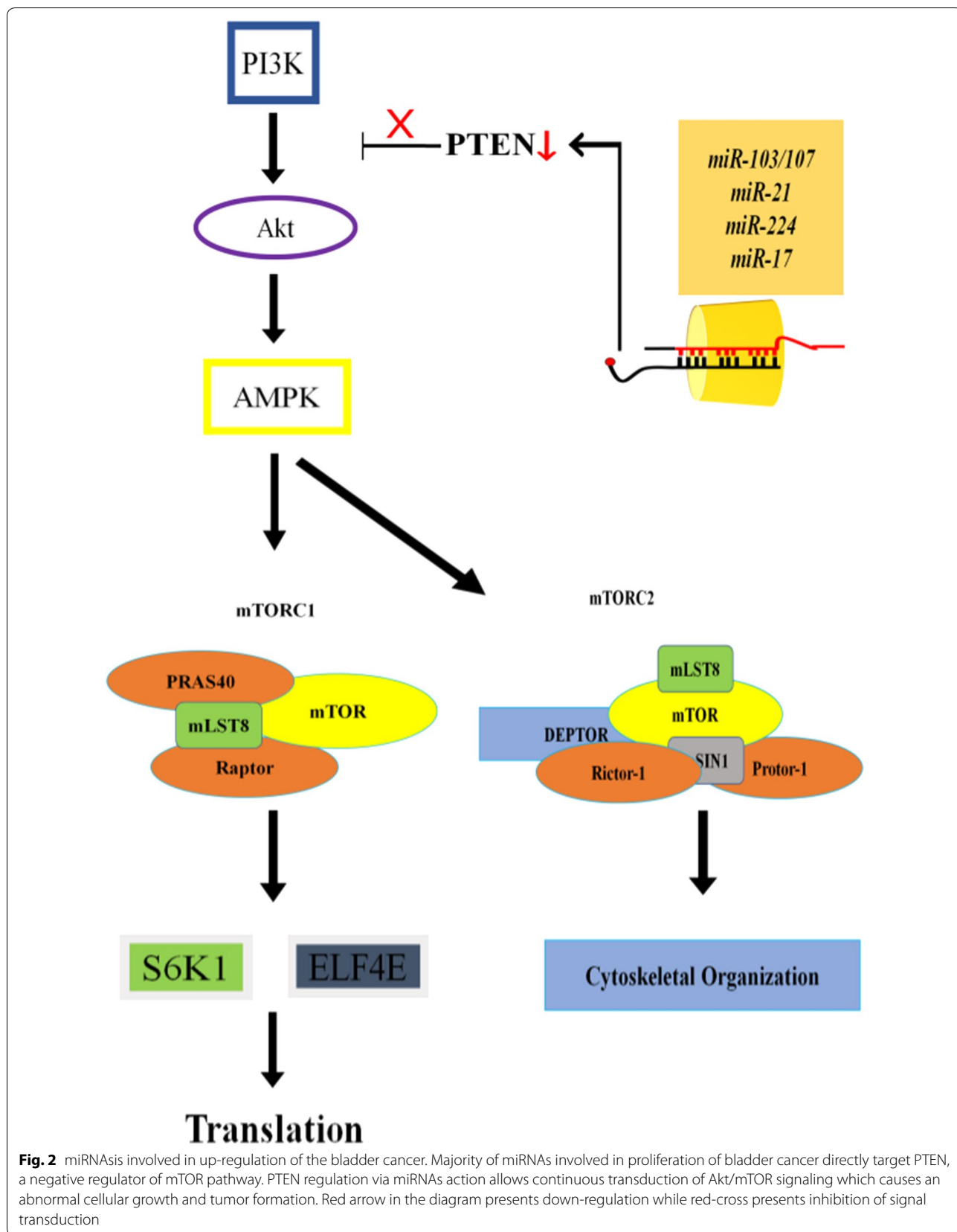


Fig. 2 miRNAs involved in up-regulation of the bladder cancer. Majority of miRNAs involved in proliferation of bladder cancer directly target PTEN, a negative regulator of mTOR pathway. PTEN regulation via miRNAs action allows continuous transduction of Akt/mTOR signaling which causes an abnormal cellular growth and tumor formation. Red arrow in the diagram presents down-regulation while red-cross presents inhibition of signal transduction

a role in enhanced BC size and growth by the activation of AKT/mTOR signaling pathway. Moreover, aberrant expressions of SPAG-5 are responsible for poor survival of BC patients [46].

mTOR signaling transduction in BC is also amplified by the up-regulated expression of sororin (CDCA5)—a protein essential for sister chromatid separation and cohesion. Its high expression has been correlated with poor survival. Sororin inhibits intrinsic apoptosis and promotes cell proliferation by activating PI3K/AKT/mTOR pathway and also by enhancing expression of cyclinD and CDC2 [47].

In physiological conditions, mTOR helps the cell's growth and metabolism through mTORC1 signaling. mTORC1, after being activated, induces the accumulation of transcription factor SREBP-1c in nucleus, which is a pre-requisite step for de novo synthesis of fatty acids and cholesterol. Both fatty acids and cholesterol are necessary for different cellular processes and growth. SREBP-1c up-regulates fatty acids synthase (FASN) expression. FASN is essential for the catalysis of final steps of fatty acids de novo synthesis and its high expression in BC is directly associated with cancer development, patients' shorter survival and higher probability of the relapsing of disease [48, 49]. Pyruvate kinase M2 (PKM-2) is recently discovered to be a modulator of SREBP-1c. PKM-2 physically binds with SREBP-1c to activate it. Its over-expression in BC has been reported by Su et al. [50]. It has been demonstrated in vitro experiment that the down-regulation of PKM-2 causes reduction in SREBP-1c expression via modulation of AKT/mTOR signaling. PKM-2 inhibition blocks the phosphorylation of AKT and mTOR which reduces SREBP-1c expression and ultimately, suppresses FASN transcriptional activation. Moreover, PKM-2 inhibition also inhibits cell growth and promotes cell apoptosis [48].

mTORC2 induces stabilization and maturation of PKCs by phosphorylating them at their turn motifs and hydrophobic motifs [51]. PKCs hyperactivation in numerous types of cancers including BC is associated with cell growth, angiogenesis and metastasis. Patel et al. treated TCCSUP cells with atypical PKC inhibitors and rapamycin and reported attenuated viability of BC cells [52]. Their study suggested that mTOR pathway plays a role in PKCs activation and also suggested a possible combined therapeutic approach for BC.

Long non-coding RNAs contribution in BC initiation and progression by modulating mTOR signaling has been also demonstrated. The over-expression of DUXAP10 in BC tissues is associated with increased cancer growth and proliferation. Knocking down of DUXAP10 in T24 and 5637 cells negatively influenced the cell growth. Mechanistically, it inhibited phosphorylation-activation

of Akt which ultimately, prevented the phosphorylation of mTOR. DUXAP10 knock-down also up-regulated expression of PTEN which induced cell cycle arrest at G1/G0 phase, inhibited cell proliferation and facilitated apoptosis by increasing expression of pro-apoptotic proteins [53].

MiRNA-mediated regulation of mTOR in bladder cancer

Several microRNAs are discovered which play important roles in modulating vital processes in cells including cellular growth and apoptosis. These miRNAs maintain the normal balance of cellular growth. Dysregulation in their expression causes uncontrolled cellular growth which then can turn into carcinogenesis. After tumor formation, miRNA also support cancer growth and metastasis [54–56]. Like is the case with other cancers, miRNA contribution in tumorigenesis in bladder has been documented as well. These miRNAs by regulating different pathways, including mTOR signaling pathway, play an important role in BC initiation, progression and metastasis (Table 1) [57, 58]. Figure 2 presents the interplay of different miRNAs in modulating mTOR signaling in BC.

MiR-103 and miR-107 are two tumor-promoting microRNAs in BC which have similar seed sequence and can only be distinguished by one base [59]. Elevated expression of miR-103/107 in BC cells facilitates the rapid cell proliferation by activating PI3K/AKT/mTOR cascade. Yu et al. inhibited miR-103/107 by transfecting antagomiR-103/107 in UMUC2 and 5637 human bladder cell lines. They have showed using western blot analysis that inhibition of miR-103/107 significantly reduced the AKT phosphorylation-activation. As a result, the activation of mTOR was also prevented which was dependent on Akt activation. miR-103/107 duo has its cell proliferating effect by blocking the expression of PI3K/AKT signaling pathway negative regulator—PTEN [60]. According to the results of Han et al. [61], reduced PTEN level in BC cells is associated with tumor aggressiveness.

Among all microRNAs, miR-99a-5p plays an essential role in modulation of mTOR/AKT signaling. It directly targets mTOR where it facilitates in the suppression of cancerous cell proliferation by binding to 3'UTR of mTOR mRNA at position 295–301 of nucleotide [62, 63]. It has been demonstrated that reduced expression of miR-99a-5p was directly associated with BC and was responsible for poor survival rate of patients [62]. The miR-99a-5p/mTOR axis induces growth inhibitory effect on BC cells by negative regulation of S6K1 phosphorylation. S6K1, a key translation regulator, is major target of mTOR and has a pivotal role in cancerous cells' growth and proliferation [64]. Another group of scientists induced expression of miR-99a-5p in 5637 and T24

Table 1 List of miRNAs and their target genes, along with their expression in bladder cancer

miRNA	Target gene	Expression	BC cell line		References
			Name	Type	
<i>miR-103</i>	PTEN	Up-regulated	UMUC2, 5637		[60]
<i>miR-146a</i>	AMPK α	Up-regulated	T24, 5637	Muscle invasive	[66]
<i>miR-21</i>	PTEN	Up-regulated	T24, 5637	Muscle invasive	[103]
<i>miR-224</i>	PTEN	Up-regulated	EJ, T24, 253 J, RT4, TCC-SUP, UMUC, J82, 5637		[68]
<i>miR-218</i>	BMI-1	Up-regulated	T24, EJ		[69]
<i>miR-222</i>	PPAR2A	Up-regulated	T24, 5637		[70]
<i>miR-99a-5p</i>	S6KI	Down-regulated	J82, HT-1376, 5637, RT4, and T24		[62]
<i>miR-100</i>	PTEN	Down-regulated	CaBER, BIU-87, 5637, T24, T4, J82, HT-1376, EJ, TCCSUP, SV4		[65]
<i>miR-126</i>	PI3KR2	Down-regulated	BLS		[71]
<i>miR-125-5p</i>	Hexokinase2	Down-regulated	T24, RT4, J82, 5637		[73]

BC cell lines by transfecting miR-99a expression-vector and demonstrated that miR-99a-5p re-expression also prevented mTORC1 and mTORC2 signaling transduction. Since mTOR is a core component of both complexes, down-regulation of mTOR causes a modulation of mTORC1 and mTORC2. These studies indicate the importance of miR-99a-5p in controlling tumor growth in BC [63]. miR-100 is another miRNA which suppresses tumor growth by directly targeting mTOR. By using the quantitative real-time PCR, Xu et al. have found that miR-100 is under-expressed in BC tissue and in BC cell lines. They further evaluated tumor-suppressor role of miR-100 by its ectopic restoration in BC cell lines and reported that its re-expression caused cell-cycle arrest and inhibited proliferation and motility of cancer cells [65].

MicroRNAs contribution in chemo-resistance and in increasing number of cancer cells is well documented. Zhuang et al. reported about the direct association of exosomal miR-146a and enhanced cancer initiating properties and chemo-resistance in BC where it has an important role by post-transcriptionally repressing AMPK α and promoting mTOR signaling. They further reported that miR-146a is highly expressed in BC cells and is secreted by cancer-associated fibroblasts at muscle invasive stage [66]. The findings of this study highlight the potential significance of miR-146a as a prognostic marker for predicting the probability of cancer recurrence and cancer stage. MiR-21 is another microRNA which has its tumorigenic influence on transitional cell carcinoma of BC by down-regulating PTEN and inducing chemo-resistance to doxorubicin. miR-21 is highly over-expressed in cancer cells and due to consequent negative-regulation of PTEN, increased rate of phosphorylation of AKT and up-regulation of anti-apoptotic protein BCL-2, cell proliferation is increased [67]. PTEN is direct target for another

microRNA-miR-224 or miR-17. The increased level of these miRNAs in BC cells correlates with lower expression of circular RNA circ-ITCH which is important for increased cell proliferation. circ-ITCH over-expression sequesters miR-17 or miR-224 and enhanced PTEN expression in BC cell lines. In vitro over-expression of circ-ITCH promoted cell apoptosis while in vivo over-expression induced suppression of xenografted tumor growth [68]. PTEN is also indirectly modulated by miR-218 in BC cells. miR-218 targets BMI-1 mRNA in non-cancerous cells but its lower expression in BC cells is the reason for BMI-1 over-expression. PTEN, being the down-stream target of BMI-1, is suppressed which allows cancerous cells to replicate. Over-expression of miR-218 inhibits BMI-1 and restores PTEN expression which consequently inhibits tumor cell proliferation and migration [69].

MiR-222 is responsible for resistance to cisplatin in BC. It is highly expressed in BC cells and decreases apoptotic response of cisplatin by activating AKT/mTOR signaling pathway and also facilitates the induction of cellular proliferation. miR-222 activates mTOR signaling by inhibiting PPP2R2A at post-transcriptional phase and its lower expression then reciprocally activates PI3K/Akt/mTOR pathway [70].

Some microRNAs have tumor-suppressing role in cancers and their expression is down-regulated in most types of cancers including BC. miR-126 is one of these microRNAs whose decreased expression in BC is associated with increased cell proliferation, migration and invasiveness [71]. Multiple targets of miR-126 have been discovered until now and their modulation by miR-126 has negative effects on BC cell viability. For instance, down-regulation of ADAM-9 mRNA causes inhibition of cell invasiveness [72] and by targeting PIK3R2 gene it modulates PI3K/Akt pathway and

has a negative impact on cell's proliferation [71]. MiR-125b-5p is another tumor suppressive miRNA. Its lower expression directly correlates with shorter survival time and metastases. Its ectopic expression in T24 and J82 cells reduced cell viability, decreased metastatic potential and promoted cell death. Luciferase reporter assay, immunoblotting and qRT-PCR suggested that hexokinase 2 might be a direct target of miR125b-5p. Hexokinase 2 is down-stream target of PI3K/Akt pathway. Western blot analysis showed that enhanced miR125-5p expression also decreases the phosphorylation activation of Akt and PI3K [73]. miR125-5p can directly or through PI3K/Akt pathway inhibit hexokinase 2 which causes tumor growth and metastasis suppression.

Zhai and Xu demonstrated that the lower levels of miR-126 in T24 cells have direct correlation with high expression of lncRNA ATB. They further reported that ATB binds complementarily with miR-126 and prevents its binding with target mRNA. KRAS is another target of miR-126 and its down-regulation by lncRNA ATB induces elevation in KRAS expression which activates AKT/mTOR signaling pathway by promoting phosphorylation-activation of PI3K, AKT and mTOR [56]. Re-expression of miR-126 by the transfection of recombinant-lentivirus vector in BLS cells facilitated apoptosis and suppressed tumor proliferation and migration [71]. This suggests the possible therapeutic importance of miR-126 in BC.

Study in 2014 reported attenuated expression of four papillary BC (pBC)-specific miRNAs. The expression of miRNAs, miR-200c, miR-205, miR-145 and miR-125 was significantly lower in high grade pBC. Further, negative correlation between miR-205/miR-200c and ZEB1/2 was demonstrated which are the downstream effector targeted for Akt [74].

mTOR pathway role in autophagy is well-documented. miRNAs also regulates mTOR induced autophagy progress. In BC this process is inhibited by miR-21 which inhibits PTEN, caspase-3, beclin-1 and E-cadherin causing cell proliferation, invasion and migration as well as inhibition of apoptosis [75]. Wang et al. reported the autophagy promoting role of sodium butyrate (NaB) in BC. NaB applied to BC cells promoted up-regulation of miR-139-5p that induced activation of autophagy via AMPK/mTOR pathway and caused overproduction of ROS. As a consequence the viability of cancer cells was reduced and the process of caspase-dependent apoptosis was activated. MiR-139-5p targets Bmi-1 which induces reversion of invasion and migration [76]. Hydroxycamptothecins initiated apoptosis via promoting AMPK/mTOR pathway dependent apoptosis [77], but the exact involvement of miRNAs in this process is not yet known.

Natural compounds as possible therapeutic solution

Natural compounds have been used for the treatment of different anomalies for thousand years [78]. Plants produce bioactive secondary metabolites that exclusively protect them from predators. These bioactive compounds have been used for the treatment of cancer as well [79]. Few derivatives of plant-based phytochemicals have gained prominence in the field of cancer biology because of their limited cytotoxic effects and high specificity. Among these bioactive compounds resveratrol, curcumin, quercetin, paclitaxel, genistein, Epigallocatechin-3-gallate (EGCG) and several others have been investigated extensively for their antitumor properties [80]. Phytochemicals have limited side effects which make them safe for the administration as proven in animal models and cell lines. These compounds interact with different molecules and signaling cascades that alter molecular landscape to tumor cells. Recently obtained data from cutting edge research have shown that deregulated signaling are critical hallmarks of BC. Accumulating scientific evidence has helped to delineate the interplay between miRNAs and natural compounds in hampering cellular growth in bladder cancer. Here we summarize few of these compounds and their interaction with miRNAs in regulation of BC and can be implemented as possible therapeutic option (Table 2).

Resveratrol

Resveratrol (chemically 3,5,4'-trihydroxystilbene) is a naturally-occurring edible poly-phenolic anti-oxidant which could mostly be found in red wine, berries, peanuts and grapes [81]. Several studies have demonstrated its chemo-preventive effects concerning tumor initiation, growth and progression [82–84]. It specifically inhibits the growth of cancer cells, leaving healthy cells unaffected [85, 86]. It triggers apoptosis by impairing oxidative phosphorylation and inducing over-production of ROS [87]. In BC cells, it induces dose-dependent inhibitory effect on Akt phosphorylation at S473. Akt inactivation negatively influences the phosphorylation of Bcl-2 agonist of cell death (BAD) at Ser112 and Ser136. Resveratrol also induces Akt phosphorylation reduction and increases phosphorylation of p38 MAPK. Since MAPK is negative-regulator of cyclin D1 and CDK, its activation results in cell cycle arrest at G1 phase. Resveratrol promotes apoptosis in BC cells by down-regulating Bcl-2 and Bcl-xL expression and by up-regulating Bax expression. It also induces cleavage of caspase-3 and PARP in a dose-dependent manner [88]. Stocco et al. demonstrated the dose-dependent inhibitory effect of resveratrol on ECV304 cells. They reported that low concentrations

Table 2 List of natural compounds and their target genes as well as their expression in bladder cancer

Natural compound	Cell line	Target gene	Expression	References
Resveratrol	T24, ECV-304	Bcl-2	Down-regulate	[88, 89]
	639Vs	mTORC1	Down-regulate	[93]
	RT4, 5637, T24	Akt, mTOR, SRC	Down-regulate	[102, 103]
	5637	Akt	Down-regulate	[105]
		PTEN	Up-regulate	
Curcumin	T24	P53	Down-regulate	[118]
	T24, J82, TCCSUP	Akt, SRC	Down-regulate	[119]
	EJ	PI3K	Suppress activation	[126]
Paclitaxel	T24	Akt	Down-regulate	[134]

of resveratrol had negligible inhibitory effect on cancer cells. On the contrary, it provided protection to cells against oxidative stress. Nevertheless, high concentrations of resveratrol modulated Bcl-2 expression and enhanced cell damage and apoptosis rate [89]. The efficacy of resveratrol cytotoxic effect is dose- as well as time-dependent. Its high concentrations (~ 150-200 μ M) induce growth arrest after 2 h of resveratrol exposure and apoptosis after 72 h of exposure in human TCC EJ cells [90]. Its time-dependent cytotoxic effect is also recorded in T24 and BTT39 cell lines too. Its cytotoxic effects include caspase 3- and 9- cleavage activation, disruption of mitochondrial membrane, cytochrome c release in cytosol and increased production of ROS [91]. Yang et al. reported that different cell lines of BC differ in their sensitivity to resveratrol. They evaluated the sensitivity of T24 and EJ cells for resveratrol and have found that despite producing similar metabolic profile, T24 were more sensitive to resveratrol. Their finding provided significant insight regarding resveratrol therapeutic potential specifically when individual differences are taken into account [92]. Schematic representation of resveratrol effects on mTOR signaling which causes changed cellular growth and tumor formation is presented in Fig. 3.

Alayev et al. evaluated mTOR signaling regulation based anti-cancer properties in BC cell line by exposing it to resveratrol and rapamycin co-treatment. They reported that treatment with resveratrol alone or in combination with rapamycin efficiently inhibited the cell growth by inducing growth stop by targeting Akt activation and preventing mTORC1 signaling cascade

phosphorylation-activation. Their findings suggested that resveratrol might be a potential option for BC treatment [93].

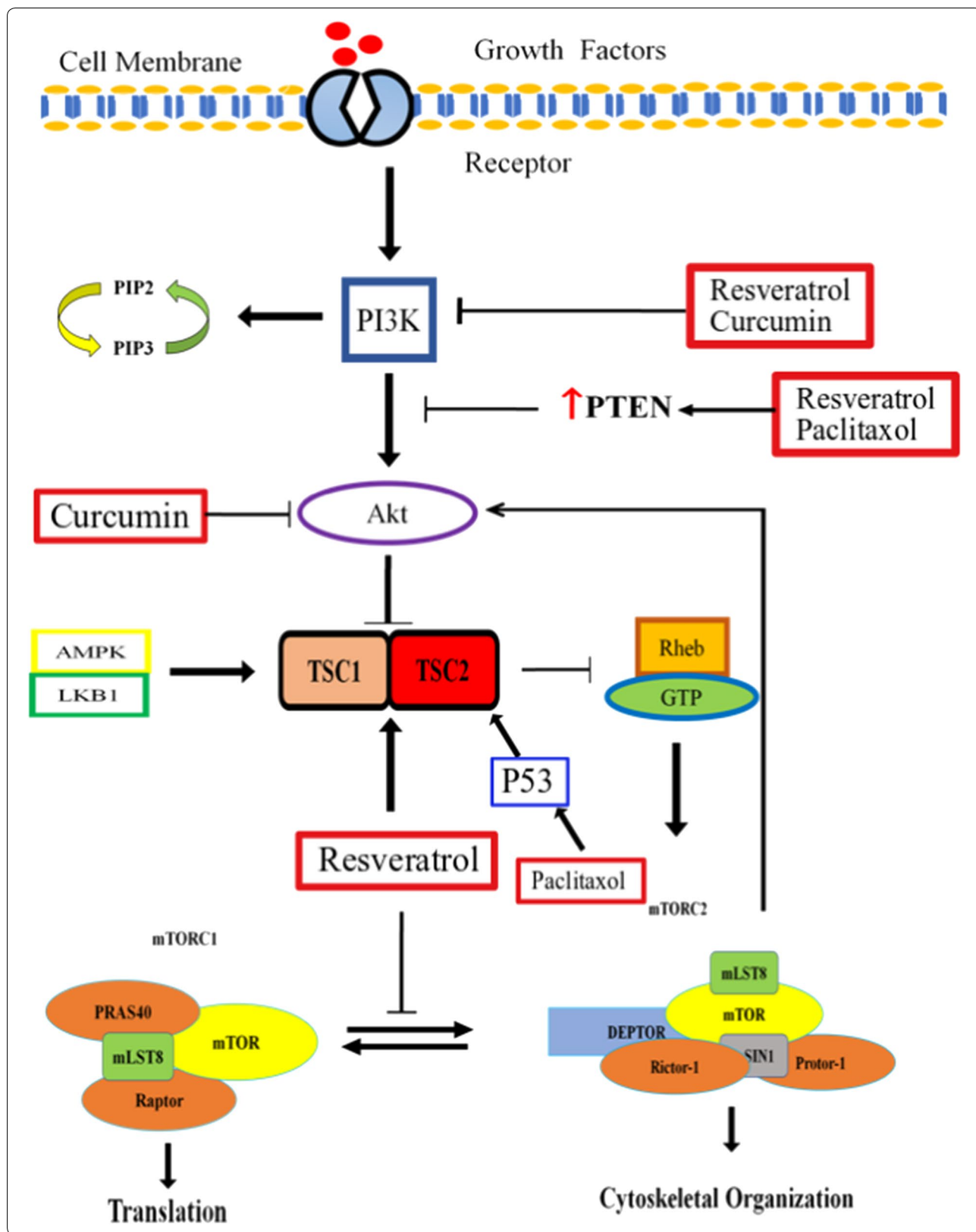
Resveratrol therapeutic potential also includes its ability to treat BC resistant to numerous drugs either by re-sensitizing cancer cells to certain drug or by inhibiting cell growth by adopting alternative apoptosis route [94–97]. It re-sensitized cancer cells to doxorubicin in a dose-dependent manner [96, 98, 99]. It has a pro-apoptotic effect on taxol resistant BC cell lines by inhibiting cell growth beyond S-phase [100]. Among several drugs investigated, Wang et al. reported that resveratrol was most efficient in inducing apoptosis in adriamycin (ADM) resistant pumc91 BC cells. They also reported that resveratrol caused significant reduction in expression of MRP-1, GST, BCL-2 and LRP and an increase in expression of Topo-II [101].

In a recent study, the effect of resveratrol in BC cells varying in TP53 gene status was analyzed. Resveratrol caused a reduction in cell proliferation promoted cellular damage in all cell lines. Downregulation of Akt, mTOR and SRC expression, regulation of DNMT1 expression and an increased rate of cell death was reported in wild type P53 cells. Cell cycle arrest at S phase occurred in mutated TP53 cells while HOXB3/RASSF1A pathway modulation along with PCNA levels reduction in nucleus occurred in the highest-grade cells [102].

Resveratrol's modulating Akt/mTOR signaling by regulating microRNA expression was investigated by Zhou et al. in BC5637 and T24 cells. According to their findings, resveratrol down-regulates the expression of

(See figure on next page.)

Fig. 3 Resveratrol, curcumin and paclitaxol affects the mTOR pathway at several stages from inhibition to PI3K to suppression of Akt signaling to prevent the mTORC1 and mTORC2 complexes. Paclitaxol also stabilizes P53 which promotes complex formation of TSC1 and TSC2. TSC1/2 complex then represses mTOR signaling. These compounds mediated inhibition of mTOR complexes results in the apoptosis and stop of bladder cancer growth



miR-21 [103]—a microRNA that induces resistance to doxorubicin treatment, prevents apoptosis and facilitates uncontrolled cell proliferation [67, 103, 104]. Resveratrol-induced down-regulation of miR-21 also caused a reduction in Akt phosphorylation which reduced cell proliferation (Fig. 4). It also down-regulated Bcl-2 protein expression and enhanced caspase-3 activation which promoted apoptosis [103]. Resveratrol also facilitates apoptosis by negatively regulating mRNA and protein levels of Akt and enhancing PTEN mRNA and protein levels [105]. Similarly, this compound also induces apoptosis in gastric cancer cells by stopping Akt-PI3K signaling via up-regulating PTEN expression [106]. However, in hepatocellular carcinoma resveratrol does not induce apoptosis by modulating PTEN expression [107]. These findings suggest possible participation of another molecule which resveratrol utilizes to modulate PTEN levels. In prostate cancer, microRNAs miR-17, miR-20a and miR-106b are targeted by resveratrol by which it up-regulates PTEN levels [108, 109].

Despite its efficient anti-cancer response, scientists face the limitation of its poor bioavailability, instability and hydrophobicity which lowers its efficiency. Current investigations are focused on overcoming these limitations by using its micro- or macro encapsulations. This technique enhances resveratrol's stability and the release of its dose is also controlled [110]. Resveratrol conjugation to gold nanoparticles is also studied with respect to its stability and bioavailability [111]. Lipid nano carriers are also investigated as transport tool for resveratrol [112]. All these methods have enhanced its stability and improved its bioavailability but further experimental endeavours are essential to first unravel these technique's benefits and second to develop efficient delivery system for these encapsulations.

Curcumin

Curcumin is a polyphenol obtained from the *Curcuma longa*. Curcumin has broad range pharmacological properties [113]. Phenolic extracts of curcumin has been reported to have anti-cancer, anti-inflammatory, antioxidant and anti-microbial properties [114]. Curcumin exerts its anti-proliferative role via modulation of the gene expression of different oncogenes which inhibit cellular growth and trigger apoptosis. Curcumin regulates the expression of several signaling cascades such as the Nuclear factor Kappa B, Akt, MAPK and others. In addition to curcumin, miRNAs also modulate the expression of key signaling pathways. For instance, in human pancreatic cells, curcumin modulates the expression of miRNA-22 and miRNA-199a*. Curcumin interacts with SP1 transcription factor and estrogen receptor and thus promotes apoptosis. miRNA-22 is up-regulated by the

curcumin while miR-199a* is down-regulated resulting in the inhibition of growth in pancreatic cancer [115]. Administration of curcumin, piperine and taurine down regulated the expression of the miR-21 and interleukin-10 in hepatocellular carcinoma patients. The anti-tumor effect of curcumin results in the regulation of the miR-21. miR-21 modulation in turn facilitates cell cycle regulation and apoptosis. miR-21 overexpression down-regulates the expression PTEN and PDCD4 protein in hepatocellular carcinoma [116]. In vivo and in vitro studies have further confirmed the anti-proliferative role of curcumin in various cancers. Curcumin has also been involved in the treatment of melanoma but further studies are required to elaborate the clear mechanism behind its pro-apoptotic effect and clinical efficacy [117]. In bladder cancer, curcumin has been reported to regulate the expression of miR-1246 which enhances radio-sensitization of tumor cells. miR-1246 under the influence of the curcumin targets P53 [118]. Curcumin has been reported to regulate epigenetic modification in breast cancer via modulation of the expression of miR-203. In bladder cancer cell lines, it was observed that curcumin up-regulated the expression of miR-203 and in turn decreased the expression of AKT2 and SRC. Down-regulation of the Akt2 and Src promoted apoptosis in bladder cancer cells [119]. These findings indicate the importance of curcumin as possible therapeutic solution for bladder cancer. Curcumin downregulates the expression of miR-7641 and thus increases the expression of p16 which in turn reduces invasiveness and increases apoptosis in bladder cancer cells [120]. Nearly 40% bladder cancers have abrogated activation of the PI3K/AKT/mTOR pathway [121, 122]. Poor survival rate is major risk factor associated with mTOR signaling aberrations in bladder cancer [123, 124]. It has been reported in the rat bladder carcinogenesis model that curcumin impedes the PI3K/Akt/mTOR signaling pathway. In addition to this the investigators postulated that curcumin directly inhibits the expression of insulin-like growth factor (IGF2) and prevents the phosphorylation of the insulin receptor substrate 1 (IRS-1) a prerequisite for PI3K signaling initiation thus blocks cellular growth and proliferation [125]. Another recent study has delineated the link between curcumin administration and mechanistic down-regulation of proto-oncogene c-MYC. The investigators suggested that curcumin in EJ bladder cancer cells prevents the activation of PI3K thus suppresses the downstream activation of mTOR and constitutive activation of proto-oncogenes such as the c-MYC [126].

The stability and bioavailability of curcumin is a major concern. These shortcomings lower its chemopreventive advantages. Like resveratrol, nano technology has assisted in overcoming its limitations but extensive

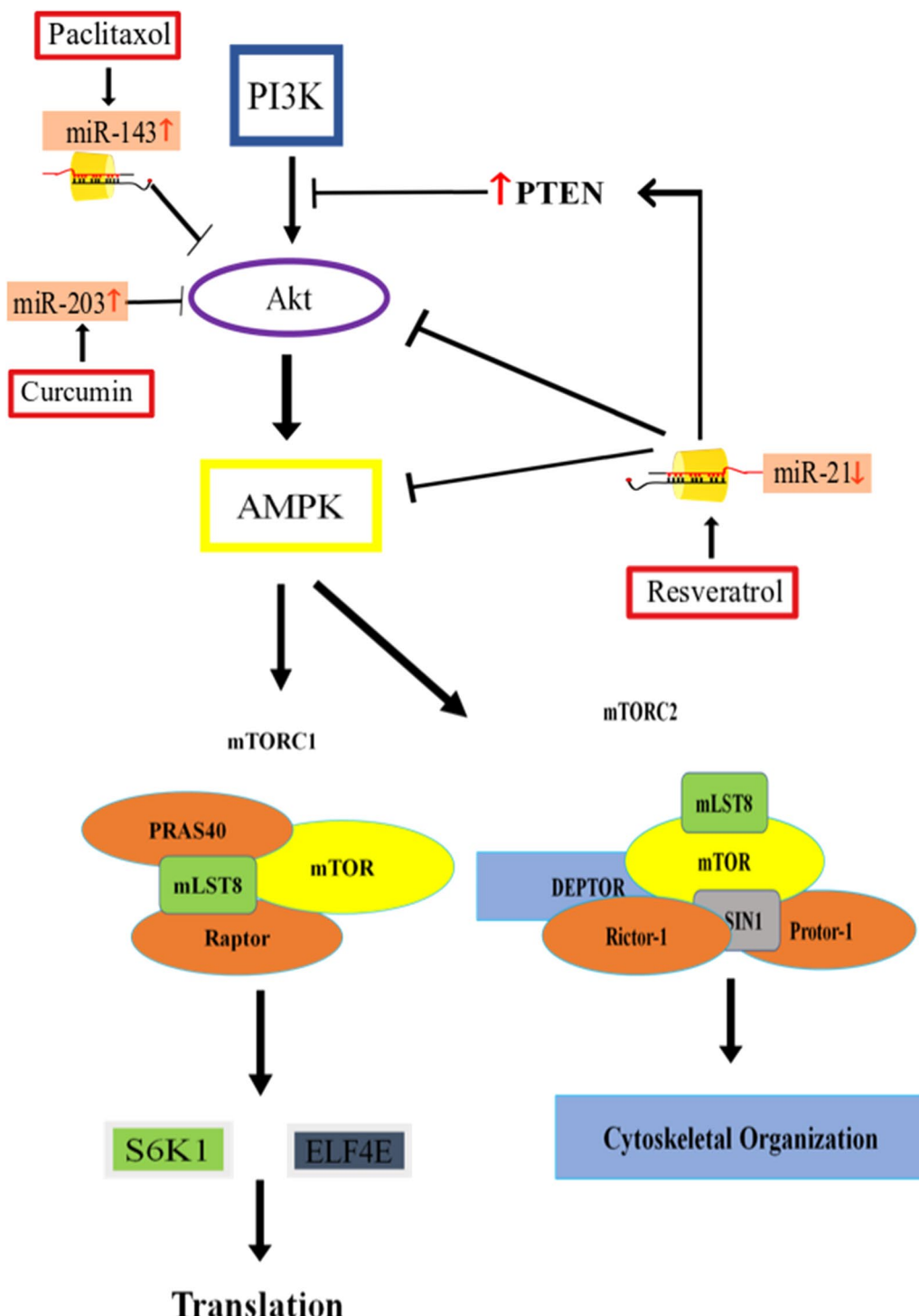


Fig. 4 MiRNAs under the influence of resveratrol, curcumin and paclitaxel efficiently inhibit the downstream signaling cascades that in turn promote the apoptosis and stop cell growth. These compounds mediated signaling up-regulates PTEN expression, inhibits Akt activation and also regulates activation of mTOR signaling-downstream effector molecules. Red up-arrow in diagram presents up-regulation while red down-arrow presents down-regulation

research is still needed to develop better delivery systems for these nano carries and to lower cytotoxicity of nano particles [127, 128].

Paclitaxel

Paclitaxel is obtained from the bark of the western yew plant and possess anti-leukemic and anti-tumor properties [129]. Several preclinical studies have demonstrated the anti-proliferative role of paclitaxel in different cancers such as the ovarian, breast, lung and sarcomas such as the kaposi's sarcoma. Paclitaxel has been employed as an adjuvant for treating tumors related to gastroesophageal, cervical, prostate, head and neck and endometrial tissues [130]. Despite its involvement as a potent chemotherapeutic agent there is one major concern regarding the administration of paclitaxel (paclitaxel resistance). This has greatly hampered its application as chemotherapeutic agent. However, miRNAs can reduce the paclitaxel resistance. It has come to limelight less lately that miR-200c can overcome paclitaxel resistance via modulation of the cathepsin L (CTSL)- mediated epithelial-mesenchymal transition in A549 cells via feedback loop system [131]. Also, paclitaxel resistance was reversed by the overexpression of miR-107 in A549/Taxol cells. Overexpression of the miR-107 significantly reduced the expression of Bcl-w in A549/Taxol cells [132]. However, interaction of paclitaxel with miRNAs in bladder cancer and regulation of mTOR still requires substantial studies. Paclitaxel in combination with the geridonin inhibited cell growth and promote mitochondrial apoptosis in gastric cancer cell lines. Paclitaxel and geridonin when administrated synergistically resulted in the up-regulation of PTEN that recruited P53 and suppressed growth of the gastric cancer cells. Activated PTEN prevented phosphorylation of the Akt and murine double minute 2 (MDM2) protein thus inhibited growth [133]. Owing to these findings' paclitaxel can be used as a therapeutic approach in synergy with geridonin for gastric as well as bladder cancer. In bladder cancer, paclitaxel up-regulates the expression of miR-143 which post-transcriptionally inhibits the expression of Akt [134].

The anti-tumor properties of these compounds in bladder cancer are well-studied but data regarding its influence on mTOR signaling modulating miRNAs require further investigations to better understand its mechanism of action. Improved understanding can facilitate their possible use in BC treatment.

Clinical trials on resveratrol, curcumin or paclitaxel in cancer

Experiment evidences on different cancer cell lines and animal models have extensively proved the chemotherapeutic and chemopreventive significance of resveratrol,

curcumin and paclitaxel. However, the efficacy of these compounds should be evaluated in human patients. Few papers in current decade have been published which highlighted the clinical use of these natural compounds in different cancers. According to these studies, the therapeutic intervention of all three natural compounds strongly depended on cancer stage and type and drug dosage and treatment period.

Curcumin clinical trials in different cancers are extensively done [135]. Clinical trials regarding curcumin treatment in bladder cancer demonstrated that 8000 mg/day of its dose for 3 months confer no side effects and has also attenuated bladder cancer lesions [136]. Likewise, its 3.6 g/day dose is pharmacologically beneficial in reducing the concentration of oxidative DNA adducts in colorectal cancer [137]. In another study, the administration of 180 mg of curcumin effectively attenuated the expression of glutathione S-transferase (GST) activity in colorectal cancer [138]. Major concern in adoption of curcumin at clinical level is its poor bioavailability. Despite this limitation, several clinical trials in numerous cancers have shown its anticancer activity [135, 139].

Paclitaxel was evaluated for single agent activity in phase 2 oncology group trials, Eastern Cooperative, A dose of 20 mg/m² was infused in 2 voluntary patients. Paclitaxel activity was reported in transitional BC cells [140]. In another study, paclitaxel was co-treated with gemcitabine in 102 advanced BC patients. In Phase 3 of treatment trial, it was reported that prolonged treatment of these drugs induced toxicity in patients but this strategy can be used as second line of treatment against metastatic BC [141]. Randomized phase III trial was conducted to analyse paclitaxel/gemcitabine/cisplatin in invasive BC. The outcomes showed that the adjuvant enhanced overall survival. But, the study was prematurely shut down due to which its actual outcomes remain inconclusive [142]. Recently, albumin-bound formulation of paclitaxel was also investigated in 199 patients in phase 2 randomized clinical trial. Paclitaxel formulation had similar therapeutic influence as paclitaxel, however, paclitaxel formulation led to more toxicity than paclitaxel [143].

The clinical trials of resveratrol are on initial phases [144]. Pharmacokinetics and pharmacodynamics of resveratrol in 6 hepatic metastasized colon cancer individuals was investigated. In this phase 1 double-blind study, 5 g dose was given for 2-weeks. The miniature quantity of compound along with high expression of caspase 3 was detectable in malignant liver tissue [145]. Another clinical evaluation was performed on 20 colon cancer patients in which 0.5 to 1 g of resveratrol dose was reported safe and effective in curbing tumor proliferation by 5% [146]. In 2015, Kjær and colleagues evaluated two doses,

150 mg and 1000 mg, of resveratrol on 66 middle-aged patients of prostate cancer in placebo controlled-randomized clinical study. They found resveratrol-treatment of no significance for benign prostate cancer [147]. In same year, phase 1 clinical study on therapeutic influence of MPX (Muscadine Naturals, Inc., Clemmons, NC), a skin extract of *Vitis rotundifolia*, on 14 patients of biochemically recurrent prostate cancer (BRPC) was published. The extract consisted of resveratrol, ellagic acid and quercetin. According to the study, 4000 mg dose of MPX accompanied no adverse effect, was effective in 50% of the patients and delayed recurrence of BRPC by lengthening the doubling time of PSA [148]. Early trial in high-risk breast cancer women showed that resveratrol promoted demethylation of tumor-suppressive RASSF-1 α gene. Contrarily to these studies, resveratrol treatment in patients of multiple myeloma led to renal toxicity and eventually renal failure [149]. This toxicity is multiple myeloma specific; yet, its treatment outcomes in human trials did not proven its much clinical significance in cancers. In colon and breast cancer, more human trials might prove successful in evaluating its full beneficial potential. Despite showing chemotherapeutic influence in bladder cancer cell lines and xerographs, its potential in human bladder cancer patients is not yet investigated.

Evidences from clinical trials indicate that these three natural compounds modulated NF- κ B signaling, STATs associated signaling and glutamine pathway [135, 150]. But none of the clinical trials investigated their regulatory influence on mTOR pathway. Therefore, current information available [125–127, 131–135] on underlying mechanism of action of these compounds is incomplete. Trials in bladder cancer are never done to analyze the therapeutic potential of resveratrol. One preliminary study regarding curcumin role in bladder carcinoma human subjects is not enough to delineate its capacity. Contrarily to curcumin and resveratrol, promising data exist for paclitaxel anticancer influence in bladder cancer. But none of the study highlighted the underlying mechanism of action of this compound. So, further experimental evaluation is necessary to understand the paclitaxel mechanism of anticancer action, to further validate curcumin potential and targeted pathway and to investigate the role of resveratrol in BC human patients.

Conclusion

Deregulation of the controlled cell signaling pathways trigger tumor progression and metastasis. Genomic and proteomic approaches can help to understand the underlying mechanisms involved in development and progression of BC. These approaches have helped in identification of novel possible anticancer treatment approaches such as miRNAs and natural compounds.

Reduced apoptosis is a characteristic of aggressive cancer cells and phytochemicals re-balances pro- and anti-apoptotic proteins to improve the efficacy of TRAIL-based therapeutics. Recent studies have started to give an insight on the role of miRNAs as regulators of cell division, metastasis and growth arrest. Therefore, miRNAs might be used as a biomarker target for various types of cancer. Additionally, natural compounds such as resveratrol, curcumin and paclitaxel seem to play a crucial role in the modulation of the multiple proteins of oncogenic pathways. The anti-tumor effects of resveratrol, curcumin and paclitaxel have been reported based upon the inhibition of the TGF/SMAD, NF κ -B, PI3K/Akt/mTOR, NOTCH and JAK-STAT pathways. Resveratrol inhibits the phosphorylation of the SMADs that in turn inhibit cell growth and metastasis. Resveratrol-mediated inhibition of mTOR has also recently been addressed. mTORC1 and mTORC2 are the two downstream effectors of the PI3K/Akt/mTOR pathway that has been modulated by the resveratrol. The consequence is beneficial effects on curbing cellular growth and invasiveness in BC. Resveratrol efficiently upregulates different tumor suppressor miRNAs in different types of cancer, and its suppressive effects on oncogenic miRNAs have also been well documented. Moreover, resveratrol in combination with the miR-99-5p prevents cellular growth in vitro. miR-99-5p, miR-100 and miR-126 are the three major miRNAs that regulate the expression of the PI3K, S6K and PTEN thus inhibiting cell growth and metastasis in BC. Curcumin has been extensively employed in the cancer treatment at least in cell lines. This has led to speculation that curcumin can be a potential therapeutic target for various cancers in near future. However, circumstantial research efforts are needed to put curcumin in to the therapeutic avenues. Curcumin has been reported to regulate the expression various miRNAs both at epigenetics and translational levels. Owing to these characteristics' curcumin based nano formulations can be employed for specific drug delivery to the target tissues. Curcumin based nanoformulations are under clinical trials and can be implemented for various cancers. However, there are several hurdles such as the dosage, bioavailability, toxicity, optimal indication and potential side effects which require attention. Resolving these issues can put curcumin on the list of possible therapeutic options for BC. Paclitaxel has been efficiently used in chemotherapy over the years but the major stumbling block regarding the administration of paclitaxel is drug resistance. New cutting-edge research has shed light on the approaches which can reduce paclitaxel mediated drug resistance and increase its bioavailability. However, this requires more in vivo and in vitro evidences. The interactions between paclitaxel, PI3K/Akt/mTOR and miRNAs in BC

provide significant insight on the pathology of disease which also open new venues for treating this cancer. Yet, more extensive studies on further elucidating these interactions are required, only than a possible and more effective therapeutic can be built.

Studies till date have demonstrated the individual influence of these natural compounds on BC. In future studies demonstrating cumulative influence of resveratrol, curcumin, paclitaxel or their derivatives could provide another possible therapeutic use of these compounds in BC. Keeping on mind the multitargeted approach of resveratrol, curcumin, paclitaxel and miRNAs and their robust anticancer effects that regulate many cellular processes, these substances could be considered an important molecular and pharmacological effective armament against BC.

Acknowledgements

Not Applicable

Authors' contributions

All authors contributed equally to the manuscript. Conceptualization, K.K., C.Q., Z.J., M.J.I., H.S., S.R., A.I., Ž.R.; validation investigation—data curation writing—all authors; review and editing, B.S., and J.S.-R. All the authors read and approved the final manuscript.

Funding

No funding received.

Availability of data and materials

Yes.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

No.

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Received: 15 October 2020 Accepted: 16 November 2020

Published online: 23 November 2020

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