**Role of AKT and mTOR signaling pathways in the induction of epithelial-mesenchymal transition (EMT) process**

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**Abstract**

Epithelial-mesenchymal transition (EMT) is a critical process in the development of many tissues and organs in multicellular organisms that its important role in the pathogenesis of metastasis and tumor cell migration has been firmly established. Decreased adhesive capacity, cytoskeletal reorganization, and increased mobility are hallmarks of the EMT. Several molecular mechanisms promote EMT, including regulation of the levels of specific cell-surface proteins, ECM-degrading enzymes, and altering the expression of certain transcription factors and microRNAs. EMT process is modulated through multiple signaling pathways including the AKT/mTOR pathway. AKT is a key component in numerous processes which was recently shown to regulate the EMT through suppression of the expression of E-cadherin via EMT transcription factors. On the other hand, mTOR complexes can also regulate the EMT through the regulation of cell’s actin cytoskeleton by altering the PKC phosphorylation state and direct phosphorylation and activation of Akt. Here we review the effect of AKT and mTOR on EMT and consequently metastasis and cell motility.

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1. A brief overview of EMT

Epithelial-mesenchymal transition (EMT) is a highly conserved, fundamental, and reversible biological process that regulates morphogenesis in multicellular organisms. The EMT allows epithelial cells to undergo substantial biochemical alteration and to achieve mesenchymal phenotypes, such as increased migratory capacity, invasiveness, and resistance to anoikis [1,2]. This process alters cell-cell and cell-extracellular matrix (ECM) adhesion and isolates epithelial cells from surrounding tissue. Functionally decreased adhesive capacity, cytoskeletal reorganization, and increased mobility are hallmarks of EMT [3]. The molecular basis of this process entails profound changes in the gene expression of proteins involved in the cell adhesion process, such as the reconstruction of cell-cell contacts and interaction with ECM [4]. Several molecular mechanisms are known to promote EMT, including regulation of the levels of specific cell-surface proteins, ECM-degrading enzymes, and altering the expression of certain transcription factors and microRNAs [3]. A number of key transcription factors, including Twist, Snail, Slug, Zinc-finger E-box-binding (ZEB) family, have been described as major drivers of the EMT program. The interconnection between these transcription factors creates a signaling network required for tumor progression [5–7]. Accumulating evidence revealed that intracellular PI3K/Akt, MAPK, and Rho GTPase cascades are key signaling mediators to activate the EMT inducing transcription factors [8]. This review aims to discuss the potential role of AKT and its downstream molecule, mammalian target of rapamycin (mTOR), in the EMT.

2. AKT and EMT

AKT, a Serine/Threonine kinase, is a key component in numerous processes, which phosphorylate and regulate vital downstream effector molecules including FOXO, mTOR, GSK3β, and many other effectors that control cell growth, proliferation, survival, genome stability, glucose metabolism, and neovascularization [9–11]. The biochemical mechanisms leading to AKT activation have recently been reviewed [9–12]. Phosphorylation of AKT occurs downstream of the PI3K, a signal transducer enzyme, which is mostly activated by growth factor receptors [13]. Activation of PI3K leads to PI3P phosphorylation and conversion to PI3P. Since the PI3P located at the cell membrane, once AKT correctly positioned at the cell membrane through binding to PI3P, AKT can be phosphorylated by its activating kinases [14]. The Akt is overexpressed in many human tumors, promoting cancer cell growth, metabolism and survival and also recent data suggest inducing EMT, angiogenesis, and metastasis [2].

3. AKT and EMT transcription factors

EMT is characterized by loss of epithelial markers, such as E-cadherin, and higher expression of mesenchymal markers, for example vimentin and fibronectin, which this event is mostly regulated by three master transcription factors, Snail (Snail1, Snail2/Slug), ZEB (ZEB1, ZEB2/SIP2), and basic-helix-loop-helix (Twist) (Fig. 1) [15–18]. Snail and Slug proteins as Zinc finger transcription factors demonstrated to be involved in EMT and implicated in cancer metastasis. Snail induces EMT through suppression of CDH1 gene which encodes E-cadherin, an important epithelial marker [19,20]. ZEB1, a member of zinc finger homeobox transcription factor, also shown to be a crucial factor which binds to E-box elements within the promoter region of the E-cadherin (CDH1) gene and inducing EMT [21]. TWIST1 belongs to the basic-helix-loop-helix (bHLH) family that is fundamental during EMT and metastasis. Besides, this transcription factor is also involved in the down-regulation of CDH1 gene expression [22].

EMT process and specially E-cadherin level and localization are modulated through multiple signaling pathways including AKT/mTOR pathway [19]. AKT activation has been shown to phosphorylate and activate the EMT transcription factors [23]. Recent studies have also emphasized the role of the AKT signaling pathway in the induction of EMT in different cancer cells. An elevated level of activated AKT in prostate cancer results in declined E-cadherin expression that, in part, is attributed to the accumulation of Snail, in the nucleus [24]. Furthermore, inhibition of AKT by PIA, an AKT inhibitor, in oral cancer cell led to down-regulation of Twist and Snail and increased expression of E-cadherin and b-catenin while reducing the migration capacity of the cells [8]. In thyroid cancer cells interleukin (IL)-8 stimulates the Akt phosphorylation leading to Slug expression which in turn induces CDH1 gene suppression [25]. Moreover, it has recently been shown the AKT might also induce expression of Twist that causes E-cadherin down-regulation hence diminish cell migration in oral squamous cell carcinoma cells and melanoma [26,27]. Expression of ZEB-1 as a repressor of E-cadherin expression can also be prompted by AKT-mediated NF-kB activation, which is consistent with another result in the role of AKT in EMT by Julien et al. [28,29].

4. AKT activating factors and EMT

4.1. Growth factors

AKT is affected by a variety of extracellular stimuli, such as growth factors, specific proteins, and dysregulation of some tumor suppressors, which can consequently affect expression of EMT transcription factors and induction of EMT. Epidermal growth factor (EGF) activate PI3K/AKT signaling pathway through EGF receptor family consisting the epidermal growth factor receptor (ErB1/EGF/HER1), ErB2 (HER2/neu), ErB3 (HER3), and ErB4 (HER4). It has been indicated that HER2 receptor is more potent in the activation of the PI3K/AKT pathway. EGF also causes EMT and invasion via ERK1/2-phospho-Smad2/3- Snail signaling pathway in breast cancer cells [30,31]. Likewise, fibroblast growth factor (FGF) signaling is involved in cancer development and invasion [32]. It has been demonstrated that fibroblast growth factor 2 (FGF2) causes an increase in expression of Slug and ZEB1 through PI3K/AKT/mTOR and MAPK/ERK signaling pathways in ovarian cancer cells [33]. Another growth factor, Insulin-like growth factor-1 (IGF-1), induces Slug and Snail mRNA expression and studies have shown that its effect is dependent on PI3K/AKT signaling [34]. Activation of PI3K/AKT signaling by hepatocyte growth factor (HGF) occurs through its c-met receptor. Activated c-Met enhances PI3K activity promoting EMT and cell motility in lung cancer cells [35].

4.2. Down-regulation of tumor suppressors

The suppressive effect of P-53, retinoblastoma (Rb) and PTEN on
tumor progression has been well established. Recent evidence has also been shown that these tumor suppressors involved in EMT and metastasis. It has been demonstrated that Down-regulation of P53 is involved in invasion and EMT through activation of the PI3K/AKT signaling pathway and by the down-regulation of E-cadherin in ovarian carcinoma [34]. The retinoblastoma (Rb) reported being inactivated by mutations in many cancers [36]. Reduced expression of Rb has been shown in invasive gastric carcinoma [37]. Dephosphorylation of Rb causes mTOR complex 2 (mTORC2) activation leading to AKT phosphorylation and invasion [38]. PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a dual lipid and protein phosphatase. Loss of PTEN function results in the accumulation of PIP3 which in turn triggers the activation of its downstream effectors, such as AKT [39]. The PTEN/PI3K pathway dysregulation is known to be involved in many cancer types [40]. Loss of PTEN function causes hyperactivation of PI3K/AKT signaling resulting in increased cell proliferation and invasion in prostate cancer [41].

### 4.3. Other proteins involved in EMT through AKT

Bone morphogenetic protein-2 (BMP-2) is a member of the transforming growth factor-β (TGF-β) superfamily and it has been shown that BMP-2 increases AKT phosphorylation. Also, Beck and his colleagues observed that inhibition of the PI3K/AKT signaling pathway leads to blockage of BMP-2-mediated migration and invasion in gastric cancer cells [42]. It has also been demonstrated that BMP down-regulates PTEN via RAS/ERK, which leads to AKT phosphorylation and activation [43,44]. Fibroblast activation protein (FAP) belongs to the serine protease family and expression of FAP has been shown in many human carcinomas [45]. Wag et al. found that a decrease in FAP expression significantly reduces phosphorylated PI3K. Furthermore, they have shown that PTEN is upregulated in FAP-suppressed cells suggesting that FAP is an upstream factor modulating the PTEN/PI3K/AKT and Ras-ERK signaling pathways in oral squamous cell carcinoma capable of inducing EMT and cell migration [46].

Calcium-binding protein A4 (S100A4) is a member of the S100 protein family. It has been proved that S100A4 can induce AKT phosphorylation at serine-473 which cause slug upregulation that consequently down-regulates E-cadherin and induce EMT in squamous cell carcinoma [47]. Another protein that is involved in EMT through AKT is prostate apoptosis response-4 (Par-4), which is widely expressed in prostate [48]. Down-regulation of Par-4 in pancreatic cancer cells has been shown to stimulate PI3K/AKT signaling pathway, which might induce EMT [49]. Knockdown of inhibitor of growth protein 5 also can cause EMT via activation of EGFR/PI3K/AKT signaling pathway in lung cancer cells [50].

### 5. AKT and MMP

Expression and activation of matrix metalloproteinases (MMPs) are elevated in cancers [51]. These proteases are well known to degrade E-cadherin [52]. It has been shown that EMT extension associated to the level of MMP-2 activity and MMP-9 secretion [53]. PI3K/AKT has been shown to positively regulate MMP-9 expression [54]. It has also been suggested that Activated PI3K/AKT maybe the possible mechanism behind up-regulating MMP-9 expression and E-cadherin degradation in head and neck squamous cell carcinoma [55]. Huang and his colleagues observed the consistent result that PI3K/AKT signaling pathway inhibition suppresses MMP-9 activation in gastric cancer [56].

### 6. miRNAs, AKT and EMT

MicroRNAs (miRs) are small, non-coding RNA molecules which have a crucial regulatory role in various cellular processes and a growing body of evidence reveal the importance of these molecules in the pathogenesis of cancer development and progression [57]. Recent studies have also reported the role of some miRs in EMT and metastasis (Table 1). miR-21 represses PTEN and human sulfatase-1 (hSulf-1) expression, which cause AKT/ERK pathways activation and induction of EMT in hepatocellular carcinoma cells (HCC) [58]. In addition, miR-130b promotes proliferation and EMT of HCC by suppressing PTEN expression [59]. Moreover, miR-92a enhances metastasis potency via suppression of PTEN gene expression and activation of the PI3K/AKT pathway [60]. A similar result has been reported in neural precursor cells [61]. In contrast,
miR-19b caused positive PTEN regulation and consequent inactivation of the AKT pathway. Therefore, miR-19b has an inhibitory effect on EMT in lung epithelial cells [62]. It has been shown that miR-92a level is inversely related to EMT and metastasis in colorectal cancer and hepatocellular carcinoma patients [63]. Shen et al. indicated that miR-612 negatively regulated cell proliferation, migration, invasion, and metastasis by suppressing AKT2 in colorectal cancer [64]. In addition, Tao et al. reported the expression of miR-612 in HCC patients is inversely associated with size, stage, EMT, and metastasis of tumor, suggesting its negative role in tumor development [65].

7. mTOR and EMT

The mTOR kinase is composed of two distinct structurally and functionally complexes, mTORC1 and mTORC2 which have an important role in the regulation of cell growth and protein synthesis such as proliferation in response to growth factors and alteration of cell survival through activation of AKT [65]. The mTOR signaling pathway plays a crucial role in the regulation of proliferation, growth, and cell survival. Recent evidence has also reported that the mTORC1 and mTORC2 signaling pathway plays a key role in the regulation of motility, invasion, and metastasis of tumor cells [66].

It has been reported that activation of mTOR signaling in infiltrated macrophages increase the invasion ability of renal carcinoma cell through the induction of EMT [67]. It has also been shown that mTORC1 inhibitor rapamycin has been suppressed scratch and chemotactic migration [68]. In the process of EMT, cancer cells undergo a substantial reorganization of the actin cytoskeleton leading to the formation of lamellipodia. It seems that the mTORC2 plays a role in the regulation of the cell’s actin cytoskeleton and consequently EMT by altering the PKC phosphorylation state [68,69]. Inhibition of both mTORC1 (Raptor) and mTORC2 (Rictor) also decreases the formation of lamellipodia [68]. Consistent with this, Gulhati and his colleagues found that pharmacologic and genetic inhibition of mTORC1 and mTORC2 significantly decrease migration and invasion in colorectal cancer through decrease activation in small GTPases, such as RhoA and Rac1 signaling [70].

The mTOR signaling pathway is activated by Transforming growth factor-β (TGF-β), which is a critical cytokine and has a role in several cellular processes [71]. TGF-β can induce EMT through activation of mTOR signaling. The mTORC2 is a major downstream effector in the TGF-β signaling pathway which directly phosphorylates Akt on Ser473 and induces EMT. It has been reported Rictor knockdown cells have impaired Snail upregulation and unable to increase MMP9 expression through AKT1 and AKT2 [72]. The mTORC2 is seeming a potential target in inhibition of EMT, prevention of metastasis and invasion of the tumor cells [73]. A similar result was reported by Serrano and colleagues indicating that integrin-linked kinase (ILK) is essential for induction of EMT through TGF-β signaling which performed its action via formation complex with the rictor of mTORC2 and proposed ILK/riotor as a potential complex in targeting EMT and metastasis [65]. It is also demonstrated that rapamycin decreases invasion and metastasis through inhibition of TGF-β-dependent EMT [72,73].

Taken together, mTOR signaling as an important pathway in the EMT process and has been proposed as a potential target in restricting invasion and metastasis of various cancer cells. Therefore, it has been suggested that using rapamycin and its analogs in combination with common cancer therapies can be a suitable therapeutic strategy.

8. Conclusion

EMT is a key process in metastasis of tumor cells, which reprograms them toward dedifferentiation and mesenchymal phenotype. This article briefly reviewed the mechanisms of the AKT signaling pathway and one of its downstream protein complex, mTOR, in the induction of EMT and metastasis. Many investigations on the role of AKT in EMT have shown that factors, stimuli, and mutation, which activate AKT, can alter epithelial and mesenchymal markers that increase the migratory capacity and invasion of tumor cells. Evidence also has shown the important role of mTOR complexes in the induction of EMT and driving the cells toward invasiveness. The growing knowledge of AKT and mTOR role in the metastasis is leading to many focuses on using their inhibitors, such as rapamycin, in inhibition of EMT and metastasis. In conclusion, these signaling pathways seem a potential therapeutic target in the inhibition of EMT, metastasis, and invasion in tumor cells.

Conflicts of interest

The authors have no financial conflicts of interest.

Contributions

MS, MK and AS designed the study; AA searched for the literature. The eligible papers were read, tagged and summarized by (MK and AA) and then verified by the third reviewer (AS). MK and AS wrote the draft of the paper. MK and KKR reviewed the paper and made revisions. Any disagreement was resolved by another reviewer (MS), who was also responsible for the supervision of the research.

Acknowledgment

This study was supported by grants awarded by the Mashhad University of Medical Sciences, Mashhad, Iran (grant no. 970217).

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