

Review

uPAR: An Essential Factor for Tumor Development

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Received: 2021.05.03; Accepted: 2021.10.02; Published: 2021.10.17

Abstract

Tumorigenesis is closely related to the loss of control of many genes. Urokinase-type plasminogen activator receptor (uPAR), a glycolipid-anchored protein on the cell surface, is controlled by many factors in tumorigenesis and is expressed in many tumor tissues. In this review, we summarize the regulatory effects of the uPAR signaling pathway on processes and factors related to tumor progression, such as tumor cell proliferation, adhesion, metastasis, glycolysis, tumor microenvironment and angiogenesis. Overall, the evidence accumulated to date suggests that uPAR induction by tumor progression may be one of the most important factors affecting therapeutic efficacy. An improved understanding of the interactions between uPAR and its coreceptors in cancer will provide critical biomolecular information that may help to better predict the disease course and response to therapy.

Key words: uPAR, tumorigenesis, proliferation, adhesion, metastasis, cancer therapy

Introduction

Tumors are the result of uncontrolled proliferation of cells in different organs. Tumor development is a multistage process, that includes the generation of primary tumors, separation of tumors from primary sites, degradation of extracellular matrix (ECM), and distant metastasis of tumors. A variety of genes play important roles in the development of tumors [1-3], including the cell surface receptor urokinase-type plasminogen activator receptor (uPAR). uPAR is highly expressed in a variety of tumor cells, and a variety of signals regulated by uPAR play significant roles in tumor cell proliferation and metastasis, tumor-related glycolysis, the tumor microenvironment and angiogenesis [4-6]. Studies have found that some specific drugs and antibodies have unique inhibitory effects on uPAR. This review intends to deliver an overview of current knowledge about the role of uPAR in cancer progression and attempts to provide a theoretical

basis for tumor therapy.

Structural characteristics of uPAR

uPAR, also known as CD-87, was discovered by Vassalli et al. in 1985 [7]. uPAR is a cysteine-rich glycosylated single-chain protein with a relative molecular weight of 50 kD-60 kD [8]. uPAR encodes a protein of 335 amino acids comprising 22 amino acids (secreted signal peptides) at the N-terminus and 30 amino acids at the C-terminus, which is bound to the cell membrane via a glycosyl phosphatidyl inositol (GPI) anchor [9] (Fig. 1A). As reported, uPAR consists of three domains ranging in size from 81 to 87 amino acids, namely D1, D2 and D3 [10], which are connected by short linker regions [11, 12] (Fig. 1B). The D1 block binds to urokinase-type plasminogen activator (uPA), the D3 region anchors uPAR to the membrane surface via a GPI, and the D2 sector joins the D1 and D3 sectors together. The N-terminus of the

arginine-glycine-aspartic acid (RGD) or somatomedin-B (SMB) structure binds to the hydrophobic chamber of uPAR [13, 14] (Fig. 1B).

Transmembrane glycerophosphodiesterase GDE3, as a GPI-specific phospholipase C, cleaves and releases uPAR from the cell membrane surface to produce the soluble type of uPAR (suPAR) [9, 15, 16]. suPAR contains the ligand binding sites of uPAR and is present in plasma, urine, blood, serum and cerebrospinal fluid [17]. uPAR cleavage results in the hydrolysis of the specific SMB-binding site between D1 and D2 in the uPAR structure [18]. Consequently, there are three different structural forms of suPAR: the complete D1+D2+D3 structure, the D2+D3 structure and the free D1 fragment [11, 12, 19-21] (Fig. 1C).

The uPA/uPAR system

The uPA/uPAR system is composed of uPA, uPAR, plasminogen activator inhibitor-1 (PAI-1), endogenous plasminogen activator inhibitor-2 (PAI-2) and plasminogen [4, 15]. PAI-1 and PAI-2 exhibit inhibitory action on the uPA/uPAR system (Fig 1C). The trimer complex formed by the binding of uPA and uPAR with PAI-1 can be recognized by lipoprotein receptor-related protein and endocytosed into cells. The uPA system regulates the interaction between cells and the ECM through proteolytic cascade reaction and further regulates cell signal transduction [22]. As ligands of uPAR, uPA and vitronectin can simultaneously bind to uPAR at different binding sites [20, 23]. uPA is a single-chain protein with a molecular weight of 54 kD that contains an N-terminal domain with an EGF-like sequence, through which uPA can bind to the three domains of uPAR by forming a large hydrophobic cavity [24-26]. Vitronectin, a viscous glycoprotein

with a molecular weight of 75 kD, is widely found in blood and ECM and interacts with different kinds of ligands [27, 28].

The role of uPAR in tumors

As early as 1991, Os-sutski et al. discovered that uPAR is closely related to cancer [29]. In recent years, with the help of positron emission tomography (PET) imaging, various studies have reported that the expression levels of uPAR in patients with breast cancer, prostate cancer, bladder cancer and colorectal cancer are significantly higher than those in normal tissues [30, 31]. Moreover, patients with a higher expression level of uPAR have a lower survival rate and poorer prognosis than those with lower expression [32]. Knocking out the uPAR gene in mice leads to G2/M arrest, thereby inhibiting cell proliferation [33]. In contrast, overexpression of the uPAR gene results in the promotion of tumor cell proliferation, migration, invasion and adhesion [4]. Therefore, uPAR plays an important role in tumorigenesis and development. This review mainly describes the expression of uPAR in tumors and the important roles of the uPAR signaling pathway in tumor cell proliferation, cell adhesion, metastasis, glycolysis, the tumor microenvironment and angiogenesis (Fig. 2).

Expression of uPAR in cancer

uPAR expression is elevated during inflammation and tissue remodelling and in many human cancers [4], including prostate cancer [34-36], bladder cancer [37, 38], colon cancer [39], breast cancer [40, 41], melanoma [42], brain cancer [43], lung cancer [44], renal cell carcinoma [45], liver cancer [46, 47], gastric cancer [48, 49], ovarian cancer [50, 51], head and neck cancer [52], cervical cancer [53] and pancreatic cancer

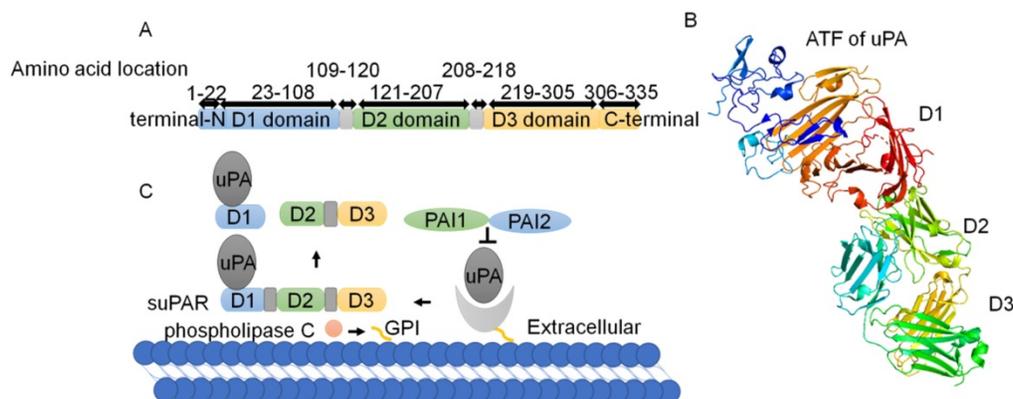


Figure 1. Two dimensional (2D) and three dimensional (3D) structures of uPAR. (A) uPAR encodes a protein of 335 amino acids comprising 22 amino acids (secreted signal peptides) at the N-terminus and 30 amino acids at the C-terminus. uPAR consists of three domains ranging in size from 81 to 87 amino acids, namely, D1, D2 and D3, which are connected by short linker regions. **(B)** The 3D structure of uPAR, with domains colored as in part (Protein Data Bank identifier 3BT2). **(C)** PAI-1 and PAI-2 exhibit inhibitory action on the uPA/uPAR system. Phospholipase C cleaves and releases uPAR from the cell membrane surface to suPAR, and uPAR cleavage results in hydrolysis of the specific SMB-binding site between D1 and D2 in the uPAR structure. Consequently, there are three different structural forms of suPAR: the complete D1+D2+D3 structure, the D2+D3 structure and the free D1 fragment.

[54]. Sustained high expression of uPAR is associated with the growth and metastasis of cancer cells [55, 56]. Moreover, uPA is also highly expressed in invasive tumors [57]. The uPA/uPAR interaction can promote the expression of oncogenes and cell proliferation, eventually leading to the development of tumors [58]. Knockout of the uPAR gene in tumor cells with the CRISPR/Cas9 system results in the inhibition of cell proliferation, migration and invasion [59]. A decrease in uPAR expression on the cell surface mitigates the development of hallmarks of cancer caused by PIK3CA and KRas mutations in colorectal cancer [60]. By interacting with uPA and IGF1R, uPAR is able to enhance the malignant potential of triple-negative breast cancer [41]. More importantly, high expression of uPAR is closely related to a poor prognosis [61]. In addition, studies have demonstrated that the decrease in the suPAR concentration after resection in patients with colorectal cancer [62] and pancreatic cancer [54] is associated with reduced mortality risk. Therefore, the expression level of uPAR can be assessed as a marker of tumor malignancy [30, 59, 63].

Regulatory network of uPAR in tumor cell proliferation

Since uPAR lacks transmembrane or intracellular domains, it needs to interact with transmembrane receptors and complexes to trigger downstream signaling and promote tumor cell proliferation [4]. Recently, Wang K et al. [59] and Semina EV et al. [64] knocked out uPAR with the CRISPR/Cas9 system, successfully resulting in suppression of human cancer cell proliferation. Silencing uPAR can inhibit the expression of the MMP2, MMP9 and P-ERK proteins in oral and tongue squamous cell carcinoma and attenuate cell proliferation [65]. Research shows that

the uPA-uPAR- $\alpha 5\beta 1$ integrin complex can bind to G-protein-coupled receptors (GPCRs) to transmit signals and promote tumor cell proliferation [66]. The interaction of the uPA-uPAR- $\alpha 5\beta 1$ integrin complex with EGFR enables the phosphorylation of Tyr397 and the Src homology 3 domain (SH3) in the intracellular domain of integrin $\alpha 5\beta 1$; this leads to the activation of focal adhesion kinase (FAK, also known as PTK2) [67-69], which results in the activation of Ras and the expression of mitogen-activated protein kinase (MAPK). uPAR can also transactivate EGFR, mediating the uPAR-initiated mitogenic signal [70, 71] (Fig. 3A). The D2A motif in domain 2, which is as effective as EGF, can promote phosphorylation of EGFR and activation of the MAPK signaling pathway, thus facilitating cell proliferation [71]. D1 of uPAR is crucial for EGFR activation, and FAK links integrin and EGFR signaling. Inhibition of EGFR kinase blocks uPAR-induced ERK signaling, implicating EGFR as an important effector of the pathway [69] (Fig. 3B).

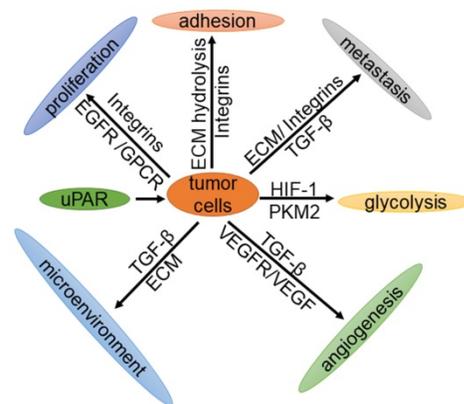


Figure 2. The function of tumor cells is regulated by uPAR. uPAR regulates the proliferation, metastasis, adhesion, glycolysis and angiogenesis of tumor cells through cell signaling, and plays an important role in the tumor microenvironment.

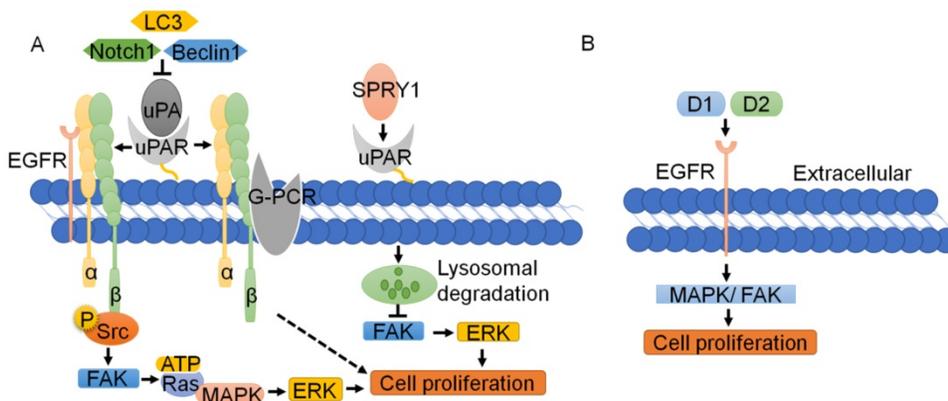


Figure 3. Function and regulation of uPAR in tumor cell proliferation. (A) The interaction of the uPA-uPAR- $\alpha 5\beta 1$ integrin complex with EGFR enables the Src homology 3 domain (SH3) in the intracellular domain of integrin $\alpha 5\beta 1$; this leads to the activation of FAK, which results in Ras activation and MAPK expression. The uPA-uPAR- $\alpha 5\beta 1$ integrin complex can bind to GPCR to transmit signals and promote tumor cell proliferation. SPRY1 can interact with uPAR and promote its lysosomal-mediated degradation, resulting in inhibition of the activation of the FAK and ERK pathways, which suppresses the tumor proliferation induced by uPAR. (B) D1 and D2 of uPAR are crucial for EGFR activation, which is as effective as EGF in promoting MAPK and FAK, and cell proliferation.

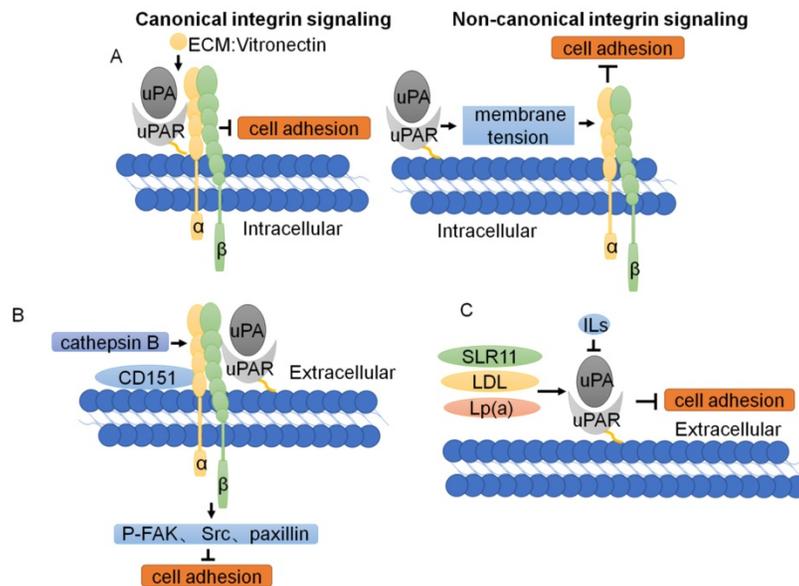


Figure 4. Function and regulation of uPAR in tumor cell adhesion. (A) Two modes of uPAR signal regulation. In canonical signaling, integrins engage the specific ligands in the ECM. In non-canonical integrin, uPAR-mediated cell adhesion, through the plasma membrane, transmits a mechanical stimulus to the integrin that signals independently of ECM binding. (B) The downstream signaling cascade of uPAR/CD151/ α 3 β 1 integrin shows that phosphorylation of FAK, Src and paxillin is reduced with knockdown of cathepsin B, uPAR, and CD151. (C) Other cellular proteins regulate tumor cell adhesion through uPAR.

Other signaling pathways are also involved in the proliferation mediated by uPAR. The Notch pathway is a highly conserved cellular signaling system that regulates the differentiation of a variety of cells and plays important roles in carcinogenesis. As reported, silencing Notch1 can inhibit the expression of uPA and its receptor uPAR, thus inhibiting the proliferation of cancer cells [72]. SPRY1, an inhibitor of the Ras-MAPK pathway, can interact with uPAR and promote its lysosomal-mediated degradation, resulting in inhibition of the activation of the FAK and ERK pathways, which suppresses the tumor proliferation induced by uPAR [73-75]. Knockdown of LC3 and Beclin-1 leads to inhibition of uPAR/integrin- β 1/Src signaling pathways, thereby suppressing cancer cell proliferation and colony formation [76]. Loss of uPAR inhibits the PI3K/AKT pathway, while downregulation of uPAR leads to upregulation of P-ERK and forces cells to use the ERK pathway as an alternative pathway for growth and survival [77] (Fig. 3A). In short, uPAR typically binds to cell membrane surface proteins, such as integrin, EGFR and GPCR to promote cell proliferation, whereas binding of uPAR by its inhibitors leads to lysosome-mediated degradation of the uPAR, thus repressing of uPAR on the proliferation of tumor cells.

Effect of the uPAR signaling pathway on tumor cell adhesion

In addition to the regulation of cell proliferation by uPAR described above, uPAR also regulates cell adhesion [77, 78]. Changes in the physical properties, composition, expression and regulation of the ECM

are considered to be abnormal signals that alter tumor cell adhesion. uPAR can regulate cell adhesion by promoting ECM proteolysis and transmitting intracellular signals [4]. uPAR regulates cell adhesion by binding directly to vitronectin and by forming complexes with integrins [79]. Recent studies have shown that cleavage of vitronectin by uPA displays a remarkable receptor dependence and requires concomitant binding of both uPA and vitronectin to uPAR, which induces cell adhesion [13]. In contrast to canonical integrin signaling, uPAR-mediated cell adhesion to vitronectin triggers a novel type of integrin signaling that is independent of integrin-engagement. The molecular mechanism enabling the crosstalk between nonintegrin adhesion receptors and integrins is dependent on membrane tension [80] (Fig. 4A).

As mentioned above, the change in tumor cell adhesion is regulated by uPAR expression. Overexpression of uPAR can strongly upregulate MMP expression and enhance breast cancer cell adhesion [78]. sLR11 regulates the hypoxia-enhanced adhesion of hematopoietic stem and progenitor cells (HSPCs) via an uPAR-mediated pathway [81]. LDL and Lp(a) lipoproteins increase the expression of uPA and uPAR on monocytes, affecting plasmin generation and monocyte adhesion. The cytokines IL-4, IL-10 and IL-13 induce a decrease in uPAR expression and lead to a change in tumor cell adhesion [82] (Fig. 4C). Studies on the downstream signaling cascade of uPAR/CD151/integrin α 3 β 1 have shown that phosphorylation of FAK, Src, and paxillin and expression of the adaptor cytoskeletal

proteins talin and vinculin are reduced with knockdown of cathepsin B, uPAR, and CD151 [83] (Fig. 4B). Collectively, these data demonstrate that uPAR regulates tumor adhesion through complex mechanisms.

Effects of the uPAR signaling pathway on tumor metastasis

uPAR is widely expressed on the surface of endothelial cells, fibroblasts and a variety of malignant tumor cells and exerts functions in cancer cell migration and tumor metastasis [14]. As reported, uPAR regulates malignant tumors through integrins on breast cancer [84] and pancreatic ductal adenocarcinoma cells [85]. Different studies have stressed that the regulatory effects of uPAR on tumor metastasis through other signaling pathways, such as those related to the ECM, integrins, and TGF- β 1.

ECM

Since uPAR lacks transmembrane and intracellular domains [4], it needs to interact with transmembrane receptors, such as ECM receptors and integrins, to activate intracellular signals. The ECM is required for cell movement and is a physical barrier to cell movement. Cell migration often involves the decomposition of ECM proteins [86]. uPAR activates a variety of intracellular signaling pathways that promote cell invasion by regulating ECM proteolysis and synergistic actions with transmembrane receptors [4]. In tumor tissues, the interaction of uPA and uPAR leads to proteolysis of the ECM through a cascade reaction. After uPA binds to uPAR, the inactive pro-uPA precursor is transformed into active uPA. Then, uPA cleaves inactive plasminogen into active plasmin, which further cleaves and activates downstream matrix metalloproteinases (MMPs) [87]. The fibrinolytic proteases and MMPs formed after activation will hydrolyze ECM and release active EGF, which promotes tumor invasion and metastasis [88]. uPAR can also degrade ECM through the proteasome pathway and activate MMPs to degrade ECM and activate EGF to further regulate the cell membrane ECM interaction, in addition to enhancing cell migration and signal transduction through the binding of and interaction between vitronectin and integrins [86, 89]. VEGF165 interacting with its receptor VEGFR-2 rapidly induces pro-uPA activation that is dependent on a change in integrin affinity, activation of MMP-2 and pro-uPA being bound to its surface receptor uPAR [90]. Taken together, uPAR is an important ECM proteolysis protein that regulates the interaction between cells and the ECM as well as cell migration [4] (Fig. 5A).

Integrins

Integrins are important cell adhesion receptors and play substantial roles in the progression of tumor metastasis [91]. uPAR and integrins form stable complexes that both inhibit native integrin adhesive function and promote adhesion to vitronectin via a ligand binding site on uPAR [92]. The uPAR and integrin α 5 β 1 interaction promotes tumor cell migration. uPAR is required to activate integrin α 5 β 3 in podocytes, promoting cell motility and activating the small GTPases Cdc42 and Rac1. Blockade of integrin α 5 β 3 reduces podocyte motility *in vitro* and lowers proteinuria in mice [93]. The uPA-uPAR-integrin α 5 β 1 complex drives activation of the GTPase Rac and actin assembly. Actin protrusions from the cell wall extend forward, and pericytes outside the cytoplasmic membrane protein undergo a decomposition of pericyclic proteins, which eliminates the ECM barrier outside the cell membrane and membrane processes [94]. The glycolytic enzyme alpha-enolase (ENO1) also acts as a plasminogen receptor, controls integrin α 5 β 3 expression and upregulates pancreatic cancer invasion, and metastasis [85]. The major downstream uPAR/integrin signaling (especially β 1 and β 3) involve activation of Src, PI3K/AKT, and MEK/ERK1-2 pathways [26]. Furthermore, uPAR cooperates with integrin complexes containing integrin β 3 to drive formation of the p130Cas-CrkII signaling complex and activation of Rac, resulting in a Rac-driven elongated-mesenchymal morphology, cell motility and invasion [95]. uPAR interaction with vitronectin initiates a p130Cas/Rac-dependent signaling pathway, leading to actin reorganization and increased cell motility [96]. Activated Rac can also stimulate actin polymerization, leading to the assembly of filamentous myosin, and ultimately stimulate membrane processes, leading to cell migration and invasion [96]. In addition, integrin-uPAR signaling can lead to the phosphorylation of Fos-related antigen-1 (FRA-1), promoting the invasion of breast cancer cells [84] (Fig. 5B).

TGF- β and EMT

Tumor cell metastasis typically requires activation of TGF- β 1 to control physiological processes [97] TGF- β signaling through mitogen-activated protein kinase, c-Jun-NH2-kinase, p38, PI3K, and G-proteins may be responsible for some of the oncogenic effects that occur in tumor cell migration and invasion [98]. TGF- β 1 induces invasion in malignant meningioma cells with an associated upregulation of uPA, uPAR, cathepsin B and MMP-9, and activation of intracellular signals of the H-RAS, ERK/PI3K, XIAP and MAPK pathways [99-101]. In

addition, TGF- β , MMPs and the uPA/uPAR system can induce epithelial-mesenchymal transition (EMT) in cancer cells [102, 103]. Interestingly, TGF- β can induce MMPs expression, and MMPs can in turn activate TGF- β , promoting EMT in cancer cells [104]. uPA/uPAR expression induces EMT in tumor cells by mediating TGF- β , resulting in tumor progression and metastasis [103, 105]. Furthermore, TGF- β RII is required for TGF- β activation of JNK1 and the resulting upregulation of uPAR expression. TGF- β activates the Ras/MKK4/JNK1 signaling cascade, leading to induction of AP-1 activity, which, in turn, up-regulates uPAR expression [106] (Fig. 5C). In addition, as mentioned above, uPAR expression is closely related to EMT. Recent studies have shown that uPAR upregulation in melanoma cells exposed to mesenchymal stem cell (MSC)-medium drives TGF- β -mediated EMT [107]. The transcription factor Forkhead box M1 (FOXM1) promote cancer EMT and metastasis by enhancing uPAR gene transcription [108], while uPAR downregulation inhibits cancer EMT and dysregulation EMT biomarker proteins [64, 103, 107]. TGF- β -induced uPA expression is human telomerase reverse transcriptase (hTERT)-dependent, and a positive association exists between hTERT and

uPA [101]. Taken together, it is clear that both TGF- β and uPA/uPAR collaborate in the induction of cancer-associated EMT.

Non coding RNA

MicroRNAs are small, noncoding single-stranded RNAs that negatively regulate gene expression at the posttranscriptional level. MicroRNAs can inhibit the expression of uPAR directly and indirectly in a variety of cancer types [34]. Targeted delivery of antisense-miR-21 and antisense-miR-10b coloaded in uPAR-targeted polymer nanoparticles (NPs)-treated mice show a substantial reduction in tumor growth [109]. As reported, miR-378a-5p and miR-23a promote tumor cell metastasis by upregulating the expression of uPAR [110, 111]. However, miR-324-5p, miR-193b and miR-143 can inhibit the expression of uPA and uPAR, thus inhibiting the migration and invasion of cancer cells [112-114]. Recently studies show that miR-200s regulate ECM remodeling, which trigger tumor cell invasion [115]. Taken together, uPAR is regulated by microRNAs to exert ECM remodeling, which plays an important role in the metastasis of cancer cells (Fig. 5D).

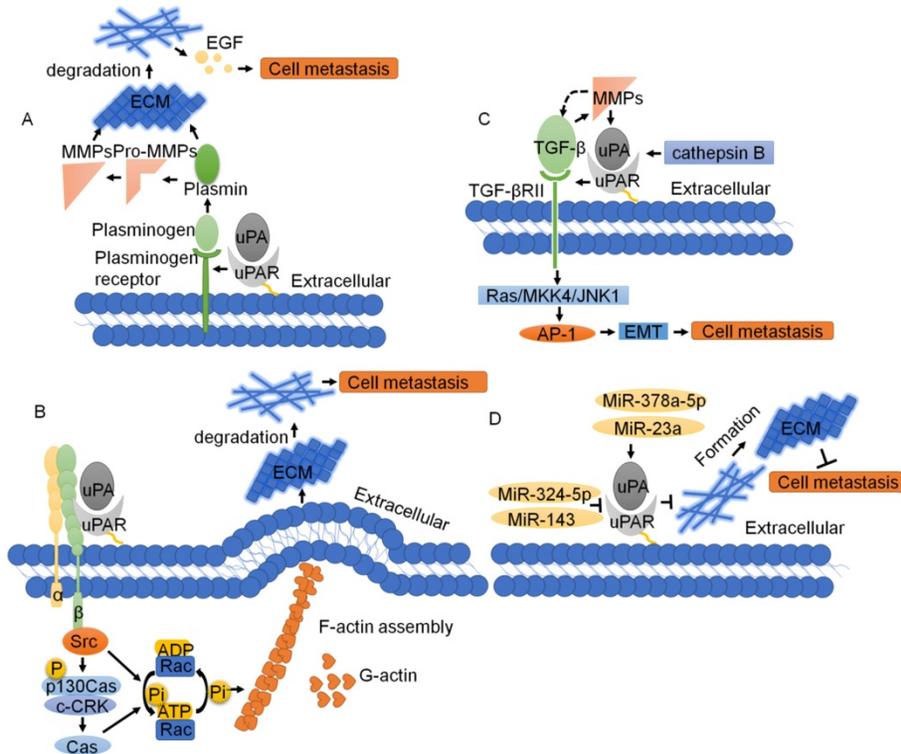


Figure 5. The regulatory network of uPAR through the ECM, integrin, TGF- β and noncoding RNA in tumor migration. (A) uPA binds to uPAR, and the inactive pro-uPA precursor is transformed into active uPA. Then uPA cleaves inactive plasminogen into active plasmin, which further cleaves and activates downstream MMPs. The fibrinolytic proteases and MMPs formed after activation will hydrolyze ECM and release active EGF, which promotes tumor invasion and metastasis. **(B)** Integrin and uPA/uPAR form the structure of the uPA-uPAR integrin complex signal, and drive the activation of GTPase Rac actin assembly, the cell wall of actin protrusions extends forward, and pericytes outside the cytoplasmic membrane protein (pericyclic protein) decomposition are eliminated outside the ECM barrier membrane. **(C)** TGF- β 1 induces epithelial-mesenchymal transition (EMT) with an associated upregulation of uPA, uPAR, cathepsin B and MMP-9. TGF- β activates the Ras/MKK4/JNK1 signaling cascade, leading to the induction of AP-1 activity, which promotes cell migration. **(D)** MicroRNAs regulate uPAR-induced ECM formation and protein degradation, which play an important role in cancer cell metastasis.

Other factors

Studies have also revealed that uPAR can interact with formyl peptide receptors (FPRs) to promote cell migration [116]. The uPAR88-92 sequence can interact with FPR1, and inhibition of uPAR/FPR1 crosstalk may be useful for the treatment of metastatic epithelial ovarian cancer (EOC) [51, 116]. Moreover, the S90P and S90E substitutions in the uPAR protein can cause upregulation and downregulation of cell migration, respectively, by mediating agonist-triggered activation and internalization of FPR1 [117], thus inhibiting tumor metastasis [116]. In addition, uPAR can enhance the metastasis and invasion induced by Ras mutations in tumor cells [118]. In human AGS gastric cancer cells, uPAR can be stimulated by prostaglandin E2 via the EP2 receptor-dependent Src/EGFR/JNK1/2, Erk1/2/AP-1, Src/EGFR/JNK1/2, and Erk1/2/NF- κ B signaling pathways, thereby promoting tumor metastasis [119]. As a co-receptor, uPAR is recycled on the cell surface and redistributed to the invasive side of cancer cells, further enhancing the migration and invasion abilities of cancer cells [4].

Different studies have stressed that uPAR has contributory effects on tumor metastasis through other signaling pathways. Silencing of uPAR inhibits the invasion and migration of oral tongue squamous cell carcinoma cells by regulating the expression of MMP2, MMP9 and p-ERK [65]. PDZ-binding kinase (PBK) can bind directly to the core region of the uPAR promoter through ETV4 to regulate the metastasis of hepatocellular carcinoma [120]. In bladder cancer, uPAR can regulate the mammalian target of rapamycin complex (mTORC) signaling pathway. uPAR silencing inhibits AKT phosphorylation at Ser473, inhibiting cell migration and invasion [37].

Regulatory network of uPAR in glycolysis

Normal cells rely on mitochondrial oxidative phosphorylation to produce ATP, while cancer cells, which are not affected by the partial pressure of oxygen, are able to gain energy via glycolysis with the stimulation of hypoxia-inducible factor (HIF-1 α) [6]. As early as 1997, Anichini E and colleagues discovered that uPAR plays an important role in glycolysis [121]. The interaction of uPA with uPAR rapidly induces the activation of glucose transporters. In recent years, studies have found that hypoxia can enhance the expression of endogenous uPAR in a HIF-1 α -dependent manner [122]. As reported, activation of HIF-1 α can upregulate uPAR expression and activate its associated signals [123, 124], while inhibition of HIF-1 α gene expression can downregulate the mRNA and protein levels of Upar [125]. Mechanistically, inhibition of uPAR with siRNA

or uncoupling of uPAR from integrin-linked tyrosine receptors (IL-TKRs) will inhibit the PI3K/AKT/mTOR/HIF1 α signaling pathway, resulting in impaired glucose uptake and a reduction of pyruvate kinase-2 (PKM2) and other glycolytic enzymes, thereby controlling the metabolism of cancer cells [6]. In addition, phosphoinositide-dependent protein kinase-1 (PDK1) can inhibit glycolysis in cancer cells [126]. Downregulation of PDK1 through the use of siRNAs targeting uPAR leads to the downregulation of downstream P-Akt [127, 128] (Fig. 6).

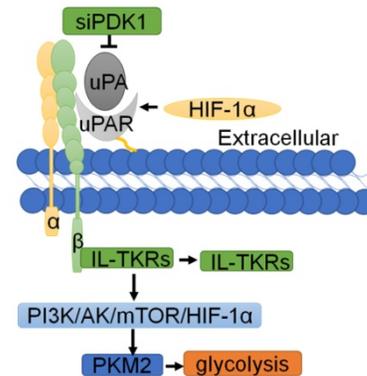


Figure 6. The regulatory network of uPAR in glycolysis. Hypoxia can enhance the expression of endogenous uPAR in a HIF-1 α dependent manner. Inhibition of uPAR with siRNA or uncoupling of uPAR from integrin-linked tyrosine receptors (IL-TKRs) inhibits the PI3K/AKT/mTOR/HIF-1 α signaling pathway, resulting in impaired glucose uptake and a reduction in PKM2 and other glycolytic enzymes, thereby controlling the metabolism of cancer cells.

Regulatory network of uPAR in the tumor microenvironment

A study focused on the microenvironment of colorectal tumors found that uPAR is expressed in macrophages, neovascular endothelial cells and myofibroblasts, and its expression is negatively correlated with survival rates [32]. Another study also showed that uPAR contributes to vascular permeability, resulting in changes in the inflammatory microenvironment in ovarian cancer [129]. In breast cancer, researchers have established a mathematical model of cancer recurrence focusing on monitoring of tissue biomarkers, including markers in the plasminogen system, and found that only the serum concentration of uPAR in cancer patients was positively correlated with cancer recurrence [130]. The interaction of uPA with uPAR activates a network of interconnected signaling pathways and induces and activates the tumor microenvironment regulatory factor TGF- β [100], which in turn promotes the expression of uPA and thus forms a positive feedback loop [131]. TGF- β function through proteolytic degradation of the ECM and regulates the expression of several MMPs and uPA/uPAR in cancer cells, thus contributing to tumor malignancy [103, 132]. uPAR

also controls the expression of the tumor microenvironment regulator IL-4 in cancer cells by activating the ERK1/2 pathway [133, 134].

Role of uPAR in tumor-associated angiogenesis

Angiogenesis plays a critical role in physiological and tumor pathological processes [135]. The blood vessels allow blood to reach all parts of the growing tumor mass, providing nutrients and oxygen, and allow invading tumor cells to reach distant sites for colonization. New blood vessels can sprout from pre-existing angiogenesis or can form by endothelial progenitor cells (EPCs) [136]. During angiogenesis, endothelial cells (ECs) degrade basement membrane, migrate through the ECM, proliferate and organize in new vessels, which can include locally recruited EPCs. uPAR activation consequent to the binding of uPA can be regarded as an “angiogenic switch” [137]. uPAR focuses on the proteolytic activity of uPA on the endothelial cell surface, thus promoting angiogenesis in a protease-dependent manner. In endothelial cells, uPAR interacts with VEGFR2, which mediates VEGF signaling and promotes angiogenesis [138]. VEGF165, the major angiogenic growth factor that initiates angiogenesis, requires coordinated proteolytic degradation of extracellular matrix provided by the uPA/uPAR system and regulation of cell migration provided by integrin-matrix interaction [66, 139]. Evidence shows that VEGF165, VEGF-E, FGF-2, EGF and HGF induced PI3K-dependent activation of pro-uPA when bind to uPAR, which leads to an increase in cell surface fibrinolytic activity [140]. Thus, uPAR represents a central mediator of growth factor-induced endothelial cell migration.

The amoeboid and mesenchymal types of invasiveness are two modes of interchangeable migration in cancer cells. A recent study showed that a role of the uPAR-integrin-actin axis in the regulation of amoeboid angiogenesis. uPAR is indispensable for ECs and ECFCs to perform efficient amoeboid angiogenesis [141]. uPAR is also functionally important in fostering angiogenesis in EPCs [142] and ECFCs [143] upon recruitment in caveolar-lipid rafts. Gangliosides and uPAR typically partition into specialized membrane microdomains called lipid rafts. The cell membrane enrichment with exogenous GM1 ganglioside is pro-angiogenic, with the opposite effect of cell membranes enriched with GM3 ganglioside. Following GM1 exogenous addition, the GM3 compartment is depleted of uPAR which is recruited within caveolar rafts thereby triggering angiogenesis [142]. Endothelial uPAR is also thought to provide a regulatory mechanism in angiogenesis.

The proangiogenic role of uPAR in ECFCs, depends on the integrity of caveolae and the presence of full-length uPAR in specialized membrane invaginations. Inhibition of uPAR expression promoted caveolae disruption. VEGF promoted the accumulation of uPAR in ECFCs caveolae in its undegraded form. VEGF-dependent ERK phosphorylation required integrity of caveolae as well as caveolar uPAR expression. Interestingly, overexpression of matrix metalloproteinase-12 (MMP-12) blocks angiogenesis by cleavage of endothelial uPAR [144], which impairs angiogenesis in SSc [145]. MMP12-dependent uPAR cleavage results into the loss of invasion properties and angiogenesis [146]. VEGF activity depends on inhibition of ECFC MMP12 production, which impairs MMP12-dependent uPAR truncation. MMP12 overexpression in ECFCs inhibits vascularization *in vitro* and *in vivo* [143]. Angiogenesis and tumor promotion are active in late stages of tumor progression by TGF- β . Evidence shows that TGF- β upregulates the expression of uPAR to regulate pro-angiogenic activity in human normal dermal MVEC [147]. Inhibition of GDF5 in TGF β -stimulated ECs impairs TGF β -dependent uPAR overproduction, impairing angiogenesis [148]. Exosomes is a new vesicular lipid transporter that is involved in various pathophysiologicals. uPAR-expressing melanoma exosomes promote angiogenesis by VE-cadherin, EGFR and uPAR overexpression and increase ERK1/2 signaling in endothelial cells [149, 150]. Tumor suppressor phosphatase and tensin homologue (PTEN) expression in endothelial cells is downregulated by uPAR to activate the PI3K/Akt pathway and support angiogenesis [151]. Mice deficient in uPAR provided an opportunity to assess the role of uPAR during angiogenesis *in vivo*. In uPAR(-/-) mice, dermal fibrosis is paralleled by endothelial cell apoptosis and a severe loss of microvessels [152]. Similarly, tumor growth of subcutaneously injected murine prostate cancer cells is significantly retarded in uPAR-deficient mice compared with wild-type mice [153]. In conclusion, uPAR plays an important role in angiogenesis *in vivo* and *in vitro*.

uPAR and cancer therapy

uPAR and chemoradiotherapy

Chemoradiotherapy (CRT) plus surgery for locally advanced cancer has recently become the standard therapeutic strategy and has a significant survival benefit compared with surgery alone [154-156]. Some studies have attempted to accurately assess CRT responses with different diagnostic approaches, but the results have mostly been

unsatisfactory. Therefore, reliable and effective biomarkers to predict the sensitivity and response of advanced tumors to CRT are urgently needed to promote individualized treatment. A recent study investigated the profiles of cytokines related to EGF and uPAR in 68 esophageal squamous cell carcinoma (ESCC) patients. The data indicated that upregulation of uPAR- and EGF-related cytokines after CRT is associated with poor progression-free survival and shortened survival [156]. The levels of EGF and uPAR for CRT in serum are reliable and predictive biomarkers for survival in ESCC patients [156]. However, the expression of uPAR is dramatically upregulated after CRT [157], and recent results suggest that PAI-1 but not uPA and uPAR might have prognostic value for patients with advanced non-small-cell lung cancer (NSCLC) undergoing radiotherapy [158]. Therefore, the response of individual tumors to CRT is highly variable.

uPAR and targeted therapy

The uPAR system regulates cell proliferation, adhesion, invasion, and migration as well as glycolysis and the microenvironment. PAI-1 mediates the endocytosis of uPAR and blocks its biological function, thus inhibiting tumor development [159]. Therefore, uPAR can be used as a marker for cancer prognosis and diagnosis and is an attractive therapeutic target [4, 160]. Quercetin has been proven to induce antimetastatic effects in gastric cancer cells by suppressing the uPA/uPAR system via modulation of various associated pathways, including the NF- κ b, PKC- δ , ERK1/2, and AMPK α pathways, indicating that uPAR may be a potential target for the treatment of gastric cancer [161]. The plant flavonoid 2',3,4',5,7-pentahydroxyflavone can effectively inhibit the expression of uPA and uPAR and inhibit TPA-induced metastasis of human breast cancer cells through the Akt/GSK-3 β /C-FOS pathway [162]. Moreover, apigenin plays an anti-invasive role by mediating the (ERK1/2, JNK)/AP-1 and (ERK1/2, JNK)/NF- κ B signaling pathways to inhibit the expression of uPAR [163]. As uPAR and FPR1 are both involved in tumor progression, an effective cell migration peptide inhibitor (Ac-d-Tyr-d-Arg-AIB-d-Arg-NH) has been synthesized to inhibit the interaction of uPAR and FPR1 to suppress migration and angiogenesis [116, 164]. Another study also stressed that inhibitors of uPAR84-95/FPR1 crosstalk may be useful for the treatment of metastatic melanoma [165]. 2G10, a recombinant antibody that binds to uPAR to form a stable complex and can block the interaction of uPA-uPAR, is effective in a xenotransplantation model of highly aggressive, triple-negative breast cancer (TNBC) [166]. Anti-

uPAR small molecules that specifically inhibit the uPAR-vitronectin interaction can inhibit cell adhesion and migration, representing a novel tool for NSCLC and colorectal cancer patients carrying Ras mutations [118].

uPAR and immunotherapy

Chimeric antigen receptors (CARs) are synthetic receptors that can alter the specificity and other functions of T cells [167, 168]. Preventing the occurrence of various diseases caused by the accumulation of cellular senescence is important for immunotherapy of tumors and other diseases [169]. Thus, CAR-T cells that counter aging-associated changes exhibit broad therapeutic potential [167, 170]. uPAR is widely expressed on the surface of senescent cells, and uPAR-targeted CAR-T cells can eliminate senescent cells *in vitro* and *in vivo* [167]. In T cells, the CAR includes an extracellular uPAR-specific ligand binding domain (scFv), an intracellular costimulatory domain (from molecules such as CD28 or 4-1BB) and a CD3 ζ T cell activation domain, which is activated by the binding of uPAR, leading to the activation and granule shedding of intracellular T cells [171, 172]. suPAR can be used as a plasma biomarker to evaluate the anti-aging activity of CAR-T cells *in vivo* [173]. Therefore, uPAR can be used as a target for CAR-T cell therapy in cancers [174] (Fig. 7). These works provide promising preliminary evidence that uPAR-directed CAR-T cells effectively target senescent cells and show that this CAR T-cell treatment has a measurable impact on disease states in immunocompetent hosts.

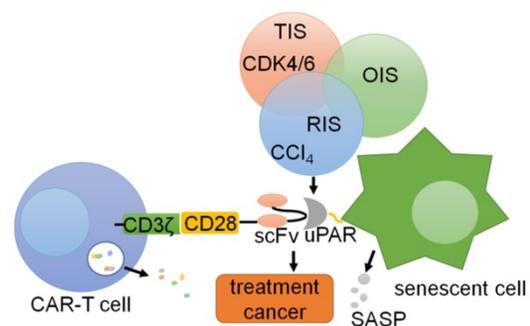


Figure 7. CAR-T cells targeting uPAR can be utilized for cells. uPAR is identified as a common upregulated marker in senescent cells in three different models: therapy-induced senescence (TIS), oncogene-induced senescence (OIS), and replication-induced senescence (RIS). Senolytic CAR T cells were generated by introducing anti-mouse uPAR scFv linked to human CD28 costimulatory and CD3 ζ signaling domains, resulting in T cell activation and degranulation.

Antibodies against uPAR can inhibit pericellular hydrolysis, thus blocking the downstream signaling pathways activated by uPAR as well as tumor growth and metastasis [175, 176]. huATN-658, a humanized anti-uPAR antibody, can significantly decrease tumor cell proliferation and metastasis [177]. Leukocyte

immunoglobulin-like receptor B4 (LILRB4) is an inhibitory immune receptor that is more highly expressed in monocytic AML cells than in normal monocytes [178]. A recent study revealed that LILRB4 can regulate different signaling pathways, including the uPAR pathway, suppress T cell activity and promote the proliferation of leukemia cells [178]. Therefore, the detection or targeting of uPAR in immunotherapy may also be of interest.

uPAR and cell drug resistance

Cell drug resistance can occur over time in the treatment of cancer and results in the weakening of drug effects, which is one of the causes of cancer-related death [179]. To reduce drug resistance, combination drug therapy has become an important method for effective treatment of cancer [180]. Exosomes are extracellular vesicles ranging in size from 40 nm to 100 nm that are often secreted by tumor cells and multiple stromal cells in the tumor microenvironment, and they can enhance drug resistance [128, 181]. The expression of uPAR is an important reason for cetuximab resistance in patients with oral squamous cell carcinoma, and combination therapy with resveratrol, which can inhibit the expression of uPAR, may provide an attractive means for treating these patients [182]. Knocking out the uPAR gene via the CRISPR/Cas9 system can reduce the resistance of tumor cells to 5-FU, cisplatin, docetaxel and Adriamycin [59]. BRAF inhibitor (BRAF-I) therapy for melanoma patients is initially highly effective, but drug resistance greatly limits its application. A recent study demonstrated that uPAR knockdown in combination with vemurafenib administration can inhibit melanoma cell proliferation by decreasing the phosphorylation of AKT and ERK1/2, and overexpression of uPAR results in reduced sensitivity to BRAF inhibition [183]. Researchers have also found that an anti-uPAR antibody (HuATN-658) combined with bisphosphonate zoledronic acid (Zometa) can inhibit breast cancer growth and bone lesions by targeting uPAR [177].

Conclusions and prospects

Tumorigenesis and progression via the uPAR signaling pathway have emerged as hot topics in the field of cancer research. uPAR is a GPI type multifunctional receptor that mainly binds to ligand molecules released by ECM hydrolysis, such as uPA and vitronectin, and then combines with integrins and G-PCR on the cell membrane to transmit the signal intracellularly [184]. This review provides an overview of emerging data, from basic research as well as cancer therapy, highlighting the evolving role

of uPAR in tumor progression. It is currently believed that uPAR expression plays an important role in tumorigenicity, and high endogenous uPAR levels are associated with tumor proliferation, advanced metastatic cancers, and glycolytic capacity [185]. uPAR has also been implicated in the angiogenesis of several solid and hematologic malignancies [186]. uPAR is aberrantly expressed through activation of signaling pathways by genetic alterations, oncogenes, transcription factors, and microenvironmental influences. Additionally, various therapeutic strategies have emerged in preclinical animal testing and clinical trials to inhibit the functions of uPAR in cancer therapy. However, the clear molecular mechanism need to be further investigated in immune escape.

Targeted uPAR immunotherapy has not achieved the desired effects in the treatment of various types of cancers. One reason for this inconsistent and poor response may be related to individual differences among patients as well as tumor heterogeneity within a single patient. Therefore, the search for targeted drugs that can inhibit the binding of uPAR and uPAR target proteins as well as other membrane proteins has become extremely important [187]. Some small molecules and antibodies that can either suppress the expression of uPAR or block the interaction between uPAR and related membrane proteins are able to inhibit the development of tumors. A combination of resveratrol and cetuximab inhibits the expression of uPAR and has been used to treat cancer [184]. Moreover, uPAR-targeted CAR-T cells can eliminate senescent cells *in vitro* and *in vivo* [167], indicating the broad therapeutic potential of uPAR in immune therapy. Coronavirus disease 2019 (COVID-19) is characterized by suppressed lung fibrinolysis. Recent studies have shown that uPA can regulate alveolar type 2-mediated re-alveologenesis [188]. The expression of suPAR is highly correlated with the characteristics of COVID-19 patients. Thus, studies of the uPA/uPAR system are helpful for identifying drugs to prevent or even treat COVID-19 [189]. As such, further analyses of immune therapy and disruption of the interactions between uPAR and its coreceptors represent an attractive strategy for targeting aggressive malignancies.

Abbreviations

uPA: Urokinase-type plasminogen activator receptor; uPAR: Urokinase-type plasminogen activator; suPAR: soluble type of uPAR; ECM: Extracellular matrix; GPI: Glycosyl phosphatidyl inositol; RGD: Arginine-glycine-aspartic acid; SMB: Somatomedin-B; PAI-1: Plasminogen activator

inhibitor-1; PAI-2: Plasminogen activator inhibitor-2; MMPs: Mitochondrial Membrane Potentials; PET: Positron emission tomography; G-PCR: G-protein-coupled receptors; EGF: Epidermal Growth Factor; EGFR: Epidermal Growth Factor Receptor; FAK: Focal adhesion kinase; MAPK: Mitogen activator protein kinase; ERK: Extracellular signal-regulated kinase; TGF- β : Transforming growth factor- β ; VEGF: Vascular endothelial growth factor; ENO1: Enzyme alpha-enolase1; EMT: Epithelial-mesenchymal transition; FPRs: Formyl peptide receptors; EOC: Epithelial ovarian cancer; mTORC: mammalian target of rapamycin complex; HIF-1 α : Hypoxia-inducible factor-1 α ; IL-TKRs: Integrin-linked tyrosine receptors; KM2: Pyruvate kinase-2; PDK1: phosphoinositide-dependent protein kinase-1; CRT: Chemoradiotherapy; ESCC: Esophageal squamous cell carcinoma; NSCLC: Non-small-cell lung cancer; TNBC: Triple-negative breast cancer; CARs: Chimeric antigen receptors; LILRB4: Leukocyte immunoglobulin-like receptor B4; Zometa: Zoledronic acid; COVID-19: Coronavirus disease 2019; EPCs: endothelial progenitor cells; ECs: endothelial cells; PTEN: phosphatase and tensin homologue; hTERT: human telomerase reverse transcriptase; FOXM1: Forkhead box M1.

Acknowledgements

This work was supported by National Natural Science Foundation of China (NSFC) grant (31801175, 81601602), Yunnan Fundamental Research Projects (202101AT070043), National College Students' innovation and entrepreneurship training program of China (202010684006), Fund of Chengdu Medical College (CYZ16-03) and Key Project of the Education Department of Sichuan Province (18ZA0162).

Competing Interests

The authors have declared that no competing interest exists.

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