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Extra View

All Roads Lead to mTOR

Integrating Inflammation and Tumor Angiogenesis

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ABSTRACT

Mammalian target of rapamycin (mTOR) is a crucial molecule in the control of cell size and proliferation; dysregulation of the mTOR pathway is commonly found in human cancers. Many cancer-promoting kinases have been identified as regulators of mTOR activity through phosphorylation and inactivation of the TSC1–TSC2 complex. Tumor-associated macrophages (TAMs) are tumor-promoting factors in inflammation-mediated tumor development, and the signaling molecules involved in TAMs-mediated tumor angiogenesis are not well understood. Therefore, it is urgent to elucidate the cross-talk between inflammatory cells and cancers and to explore the precise pathways involved in TAMs-induced tumor angiogenesis. Recently IKKβ was found to activate the mTOR pathway and to promote tumor angiogenesis through inactivation of the TSC1–TSC2 complex by phosphorylating TSC1. This finding provides critical insights into and suggests one mechanism behind inflammation-mediated tumor angiogenesis. In this extra-view, we briefly discuss the possible influence of TAMs-released proangiogenic factors on mTOR activation and propose a model of the cross-talk between tumors and TAMs in tumor angiogenesis.

ABBREVIATIONS

TSC, tuberous sclerosis complex; mTOR, mammalian target of rapamycin; TNFα, tumor necrosis factor α; IKKβ, IκB kinase β; VEGF, vascular endothelial growth factor; TAMs, tumor-associated macrophages

THE mTOR SIGNALING NETWORK

Tuberous sclerosis complex (TSC) is an autosomal dominant tumor suppressor gene syndrome that occurs in 1 in 6,000 live births and that is characterized by benign tumors (hamartomas) and malformations (hamartias) involving multiple organ systems.[1] Common clinical symptoms include seizures, mental retardation, autism, kidney failure, facial angiofibromas and cardiac rhabdomyomas.[2,3]

Over the last decade, scientists have made substantial progress on deciphering the molecular genetics underlying TSC. Two tumor suppressor genes, *TSC1* and *TSC2*, are associated with the development of TSC, and mutations of *TSC1* and *TSC2* are equally responsible for familial TSC.[1,4] *TSC1* and *TSC2* encode proteins TSC1 (also known as hamartin; 130 kDa) and TSC2 (also known as tuberin; 200 kDa), respectively. TSC1 can stabilize TSC2 by binding with TSC2, which prevents TSC2 from ubiquitination and degradation; TSC2 acts as a GTPase-activating protein to regulate the GTPase function of Ras homolog enriched in brain (RHEB) by decreasing the ratio of GTP to GDP bound on RHEB. GTP-bound RHEB activates mammalian target of rapamycin (mTOR) activity, and GDP-bound RHEB inhibits that activity, suggesting that the TSC1-TSC2 complex downregulates mTOR activity by suppressing the function of RHEB.[5,6] Accumulating evidences suggest that dysregulation of mTOR results in activation of S6K1 and inactivation of 4EBP1, further enhancing protein translation and cell proliferation; this dysregulation contributes to the development of human diseases including TSC, Peutz-Jeghers syndrome and various cancers.[7]

Multiple signaling pathways may affect tumor growth and progression by modulating mTOR activity. Growth factors, mitogens, energy, nutrients, and cellular stress

influence mTOR activity through posttranslational modification (e.g., phosphorylation) of tumor suppressors TSC1 and TSC2. Numerous kinases, including AKT,[8-10] RSK1,[11] ERK,[12] GSK,[13] CDK1,[14] p38,[15] MK2[15] and AMPK,[16] have been suggested to function as TSC kinases that integrate extracellular signals and tumorigenesis through control of the TSC1/TSC2/mTOR pathway. For instance, insulin and epidermal growth factor (EGF) regulate mTOR activity by inactivating TSC2 through AKT-mediated TSC2 Ser939, Ser981, Thr,993, Ser1130, Ser1132, Thr1462 and Ser1798 phosphorylation[8-10] and through ERK-mediated TSC2 Ser664 phosphorylation,[12] respectively. In contrast, energy depletion and hypoxia can suppress mTOR function by increasing TSC2 activity via AMPK-mediated Thr1227 and Ser1345 phosphorylation[16] and via HIF-1-driven REDD1 expression,[17] respectively.

Although emerging data indicates that regulation of the mTOR signaling network by these growth stimulators and cellular stress contributes to tumor development, it is unclear whether the inflammatory cytokines, important factors in tumorigenesis, also have roles in regulating the mTOR pathway.

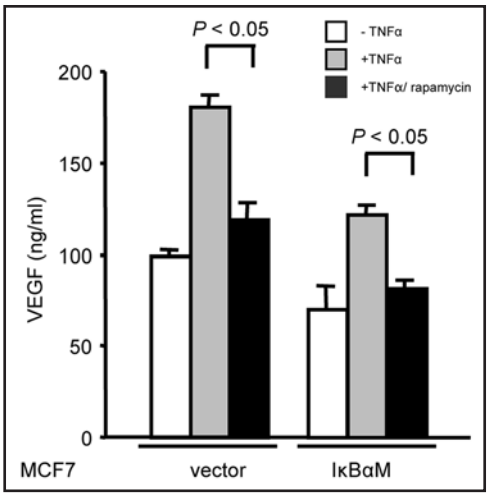

Figure 1. Rapamycin represses TNFα-induced VEGF expression in IκBαM expressed MCF-7 cells. Cells were serum starved overnight, and then they were left untreated or were pretreated with or without 5 nM rapamycin for 1 hour, incubated with 20 ng/ml TNFα for three hours, and then allowed to recover in normal medium for 24 hours. The amount of VEGF secreted was determined by ELISA.

mTOR: A SIGNAL COORDINATOR IN INFLAMMATION-MEDIATED TUMOR ANGIOGENESIS

The infiltrating inflammatory cells in human tumors were first noticed in 1863 by Rudolf Virchow, who suggested that the origin of cancer presenting chronic inflammation. Accumulating data from research into the role of inflammatory cells in tumor lesions have supported Virchow's hypothesis of tumor development and established the following concept: inflammation promotes tumor development by affecting several hallmarks of cancer, including angiogenesis.[18]

Among the cytokines and chemokines secreted by the infiltrating inflammatory cells, tumor necrosis factor α (TNFα) is considered one of the most important inflammatory factors involved in inflammation-mediated tumorigenesis. In response to stimulation by TNFα, activated IκB kinase β (IKKβ), a major downstream kinase in the TNFα signaling pathway, phosphorylates IκBα and subsequently triggers its degradation through the ubiquitin-proteasome proteolytic system. Without IκBα to retain nuclear factor κB (NFκB) in the cytosol, the liberated NFκB heterodimer translocates into the nucleus and activates its target genes. Because various NFκB target genes function as inhibitors of apoptosis and mediators of inflammation, the biologic consequence of the activation of NFκB is tumor progression.[19] However, interruption of TNFα-mediated activation of NFκB by the dominant-negative phosphorylation mutant IκBαM results in only partial inhibition of tumor development, suggesting that other signaling pathways independent of NFκB-mediated tumorigenesis may be present in response to TNFα stimulation. Study results have demonstrated that IKKβ not only presents an IκB kinase but also directly phosphorylates other targets (such as the transcriptional factor FOXO3a,[20] the de-ubiquitinating enzyme CYLD,[21] and the DNA repair mediator FANCA[22]) and impairs their physiologic functions, indicating that activation of IKKβ by TNFα could result in more widespread effects on tumorigenesis by regulating multiple downstream targets.

Studying the role of NFκB in TNFα-induced expression of vascular endothelial growth factor (VEGF) in MCF-7 breast cancer cells, we unexpectedly found that interruption of TNFα-mediated activation of NFκB by the dominant-negative phosphorylation mutant IκBαM results in only partial inhibition of VEGF production (Fig. 1). However, inhibition of mTOR by rapamycin drastically suppresses TNFα-induced VEGF expression in both parental MCF-7 cells and NFκB-inactivated MCF7-IκBα cells, indicating that the mTOR pathway has an NFκB-independent role in TNFα-induced VEGF production. Indeed, results of in vivo and in vitro studies suggested that dysregulation of mTOR signaling participates in the regulation of angiogenesis by up-regulating VEGF production, which contributes to the highly vascular nature of TSC lesions.[23]

In an attempt to investigate the relationship between the TNFα and mTOR pathways, we were surprised to find that the TNFα/IKKβ signaling pathway could activate the mTOR pathway.[24] The activities of mTOR in multiple breast cancer IKKβ stable transfectants were higher than they were in the control vector or kinase-dead IKKβ-mutant stable transfectants, and small interfering RNA (siRNA)-mediated knockdown of IKKβ caused a marked decrease in mTOR activity, indicating that IKKβ may activate mTOR in response to TNFα stimulation. While investigating which molecule in the mTOR pathway is involved in TNFα/IKKβ-mediated mTOR activation, we found that IKKβ is physically associated with TSC1. Kinase assays and mass spectrometric analyses revealed that IKKβ phosphorylates TSC1 at Ser487 and Ser511 both in vitro and in vivo. In elucidating the mechanisms underlying mTOR activation by TSC1 phosphorylation, we showed that IKKβ's phosphorylation of TSC1 alters its membrane localization and inhibits its association with TSC2, thereby activating the mTOR pathway. In vitro angiogenesis assays and an orthotopic breast cancer model revealed that phosphorylation of TSC1 by IKKβ enhances VEGF expression, angiogenesis, and tumorigenesis. Moreover, tumor immunohistochemical study results suggested that phosphorylation of TSC1 by IKKβ promotes mTOR activation and VEGF production and is associated with poor clinical outcome of breast cancer patients. Taken together, these results suggest that the inflammatory cytokine TNFα

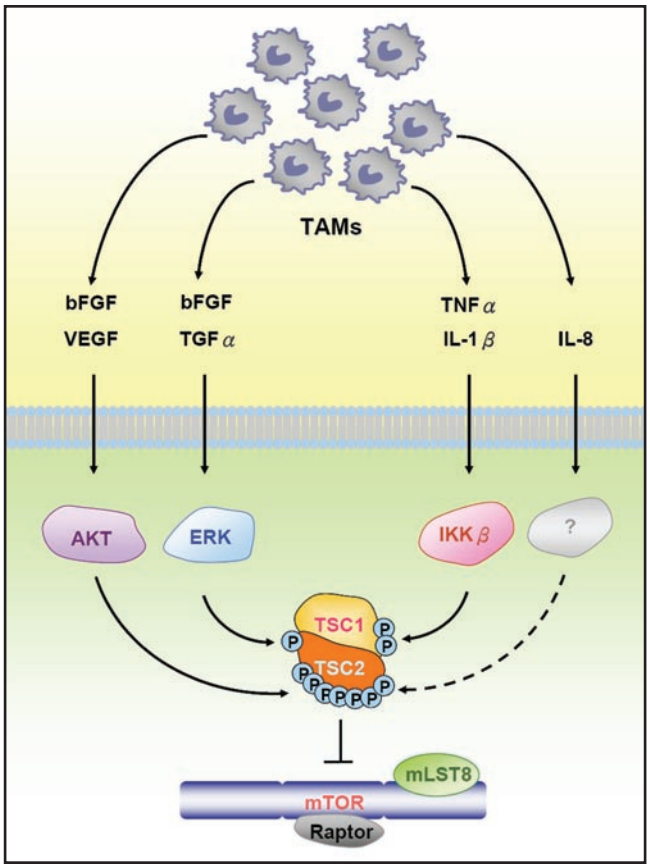

Figure 2. Diagram depicts our proposed model of TAMs-mediated mTOR activation. Several factors are associated with TAMs-induced tumor angiogenesis, including VEGF, bFGF, TGFα, TNFα, IL-1β and IL-8. We hypothesize that bFGF and VEGF induce PI3K/AKT activation, and activated AKT phosphorylates TSC2 at multiple serine and threonine sites, which destabilizes the TSC complex and further enhances mTOR activity. bFGF and TGFα also promote mTOR activation through ERK-mediated TSC2 Ser664 phosphorylation and inactivation. Furthermore, TNFα and IL-1β induce activation of the IKK complex, and activated IKKβ phosphorylates TSC1 at Ser487 and Ser511, which in turn induces dissociation of the TSC complex and culminates in mTOR activation. However, it is still nebulous whether IL-8 is involved in TAMs-mediated mTOR activation and angiogenesis.

is able to activate the mTOR pathway and enhance angiogenesis via IKKβ-mediated suppression of TSC1.[24]

TAM-MEDIATED TUMOR ANGIOGENESIS VIA DIVERSE SIGNALS INDUCES mTOR ACTIVATION

The microenvironment around tumors comprises several distinct types of cells, including fibroblasts, endothelial cells, and inflammatory cells. Among the various inflammatory cell types in and around tumors, tumor-associated macrophages (TAMs) are considered the major inflammatory cells contributing to tumor angiogenesis.[25] TAMs act like Jekyll and Hyde, releasing both proangiogenic factors [TNFα, interleukin (IL)-1β, IL-6, IL-8, bFGF (basic fibroblast growth factor), VEGF, TGFα (transforming growth factor α), EGF, and PDGF (platelet derived growth factor)] and antiangiogenic factors [TGFβ (transforming growth factor β), TSP1 (thrombospondin 1), and IFNγ (interferon γ)] to regulate tumor angiogenesis. Clinical study results have also pointed out that high infiltrating TAMs counts are significantly associated with reduced relapse-free and overall survival rates in many human cancers, supporting that the main function of TAMs is tumor promotion. TAMs release factors to promote tumor angiogenesis not only by directly enhancing the proliferation and migration of endothelial cells but also by inducing cancer cells to produce other angiogenic factors, such as VEGF, to stimulate chemotaxis of endothelial cells and promote neovascularization.

The newly identified IKKβ/TSC1/mTOR pathway provides a new view of the previously unappreciated role of mTOR in coordinating inflammation-mediated tumor angiogenesis,[24] but it is still nebulous whether other cytokines secreted by TAMs also facilitate tumor angiogenesis through the mTOR pathway. In the rest of this section, we focus on the potential effect of TAMs-released factors, especially mTOR-mediated VEGF expression, on cancer cells and list the possible mechanisms involved in this regulation (Fig. 2).

TNFα. TNFα is a proinflammatory cytokine secreted by various cells, primarily macrophages and activated monocytes. Because those types of cells are major sources of TNFα, it has been proposed that TNFα has a major function in TAMs-mediated tumor angiogenesis. In several in vitro and in vivo studies, TNFα stimulated chemotaxis of endothelial cells and promoted new capillary formation, and these angiogenic activities induced by TAMs were neutralized by antibodies to TNFα.[26] Moreover, macrophages (TAMs) cocultured with MCF-7 breast cancer cells secreted more TNFα and had a greater ability to induce vascular endothelial cell tube and network formation than did macrophages cultured alone.[27] Those angiogenic abilities induced by TAMs were inhibited by soluble TNFα receptor, indicating that TNFα is required for TAMs-mediated angiogenesis.[26] Combining these findings with ours,[24] we hypothesize that TAMs-released TNFα not only directly stimulates endothelial cells to form new capillaries but it also induces tumor cells to secrete VEGF via the IKKβ/TSC1/mTOR pathway to promote angiogenesis.

IL-1β. IL-1β is also a proinflammatory cytokine produced by a variety of cells, including macrophages, monocytes, and keratinocytes. Animal studies revealed the angiogenesis in B16 melanoma tumors was reduced in IL-1β knockout mice relative to that in wild-type mice, and B16 melanoma cells cocultured with macrophages from wild type mice secreted more VEGF and TNFα than did B16 melanoma cells cocultured with macrophage from IL-1β-knockout mice,[28] suggesting that IL-1β is a potent TAMs-derived angiogenic factor. Since IL-1β and TNFα show a marked overlap of biologic activity and they both induce mTOR activation through IKKβ,[24] IL-1β definitely facilitates tumor angiogenesis by inducing tumor cells to secrete VEGF via the IKKβ/TSC1/mTOR pathway.

bFGF. bFGF, one of 23 known members of the FGF family, is a nonglycosylated heparin-binding growth factor that is expressed in various cell types, including fibroblasts, epithelial cells, and activated macrophages. The presence of bFGF-expressing macrophages is significantly correlated with intratumoral microvessel density in pancreatic adenocarcinoma, and increased bFGF expression is also correlated with a poor prognosis.[29] Many signaling cascades, including the PI3K/AKT and RAS/MEK/ERK pathways, are activated in response to bFGF. We propose that both AKT/TSC2/mTOR and ERK/TSC2/mTOR pathways contribute to bFGF-induced angiogenesis in human cancers.

VEGF. VEGF has a prominent role in both normal and pathologic angiogenesis. VEGF is commonly expressed in activated

macrophages and various human cancers. Despite its dominant role in angiogenesis-direct promotion of endothelial cell activation and proliferation-and vascular permeability, VEGF also induces the PI3K/AKT pathway in many human tumors expressing VEGF receptors. Therefore, macrophage-released VEGF might also induce mTOR-dependent VEGF expression in cancers via the AKT/TSC2/mTOR signaling pathway, and the VEGF from these two sources may cooperate to facilitate tumor angiogenesis.

TGFα. TGFα, an EGF-related growth factor that signals through the EGF receptors, is secreted by activated macrophages, keratinocytes, and various tumor cells. In vivo studies have shown that TGFα is a potent mitogenic factor that promotes angiogenesis.[30] As a result of TGFα's signaling through EGF receptors, TAMs-released TGFα might activate the mTOR pathway through ERK-mediated TSC2 inactivation, increasing VEGF secretion from tumor cells and culminating in angiogenesis.

IL-8. IL-8 is a CXC chemokine secreted primarily by monocytes and macrophages. In vitro studies have indicated that IL-8 is involved in the TAMs-induced formation of vascular endothelial cell capillary tubes and networks. The findings of a clinical investigation on 20 patients with uterine cervical cancers suggested that the IL-8 level is significantly correlated with blood vessel density and that a high level of IL-8 is associated with infiltrating macrophages.[31] However, it is still unclear whether IL-8 has a role in mTOR activation.

In summary, some of the TAMs-associated factors perform their angiogenic function through mTOR-mediated VEGF production, but other factors might modulate tumor angiogenesis independently of mTOR. To understand this complicated and sophisticated signaling network induced by TAMs, more systematical investigations are required. Although it will be a challenge to uncover the whole picture of TAMs-induced angiogenesis, an exploration of the upstream signaling events participating in the mTOR pathway would provide a starting point for the development of new drugs for the treatment of human cancers.

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