

The structure and functions of paxillin and its roles in neovascularization

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Abstract. – Identifying and studying the molecular mechanisms of neovascularization biomarkers are critical for conquering many diseases, such as corneal diseases and cancer. Paxillin is an important cell scaffold and cellular signaling protein, especially a key molecule of the Integrin-mediated downstream signaling transduction. This review summarizes the structure and functions of paxillin, and the research progress of its roles in neovascularization. Although there are still some problems to be solved, paxillin may become an important target of anti-neovascularization therapies.

Key Words:

Paxillin, Neovascularization, Integrin, Vascular endothelial growth factor.

Abbreviations

LD: lethal dose; LIM: limit; ERK: extracellular signal-regulated kinases; MEK: Methyl Ethyl Ketone; ARF: acute renal failure; PKL: paxillin kinase linker; PIX: Proton induced X-ray; PAK: p21-activated kinase; FAK: focal adhesion kinase; MDCK: Madin-Darby canine kidney; EPCs: endothelial progenitor cells; FLK-1: Fetal liver kinase-1; VEGF: vascular endothelial growth factor.

Introduction

With the research progress on the molecular mechanisms of angiogenesis, molecular target-based treatment may bring a broad prospect to the clinic treatment of neovascularization. Normally, the cornea is in an avascular state, which is called “angiogenic privilege.” An important cause of this state is continuous corneal dehydra-

tion, which leads to the formation of tight connections between lamellar collagen structures. This anatomical structure results in failed migration of vascular endothelial cells in the cornea¹. The corneal avascular state is the key to maintaining corneal transparency and ensure good eyesight. However, a variety of corneal diseases, such as infection, trauma, and chemical injury, may lead to corneal neovascularization, which will damage the transparency and normal microenvironment of the cornea, and cause the decrease of eyesight and even blindness^{2,3}. Meanwhile, corneal neovascularization makes the intrinsic immune privilege disappear and is a high-risk factor of immunologic rejection after corneal transplantation. Currently, 4.14% of the ophthalmic patients in America suffer from corneal neovascularization⁴. The corneal neovascularization problem caused by various eye diseases, and eventually leading to blindness, has become one of most serious ophthalmic problems⁵. However, it is still lack of efficient and simple method to treat corneal neovascularization. The key biological characteristics of neovascularization are infiltration and metastasis. With the progress of cellular and molecular biology, many recent kinds of research have focused on studying the molecular mechanisms of corneal neovascularization and found that gene regulation is closely related to corneal neovascularization. Inactivation, mutation, and overexpression of certain genes play important roles in corneal neovascularization. Therefore, identifying and studying neovascularization-related biomarkers are critical for conquering the problem of neovascularization. Paxillin is a multidomain protein discovered in recent years. It is an important cell scaffold and cellular signaling protein, especially a key molecule of the Integrin-mediated downstream signaling transduction.

Through protein-protein interactions and under the regulation by phosphorylation, paxillin expression shows dynamic changes, which affects focal adhesion, cell proliferation, adhesion, migration, survival, dissemination, and cytoskeleton reconstruction. The metastasis and infiltration of new vessels are closely related to the above cellular processes. It is presumed that paxillin can also regulate the development and metastatic potential of new vessels. This review summarizes the structure and functions of paxillin and the research progress of its roles in neovascularization.

Chemical Structure of Paxillin

Paxillin is a phosphoprotein with the molecular weight of 68 kD⁶. It was firstly discovered in the fibroblasts transformed by Rous sarcoma virus in 1989. Paxillin is primarily located in local focal adhesion and it is an important component of the focal adhesion. It participates in focal adhesion assembly and can bind to focal adhesion proteins and actin. Paxillin has 11 exomes and encodes 559 aminoacids. Human paxillin is located on chromosome 12q24. The N-terminal of paxillin consists of 5 LD motifs, which are involved in protein recognition. The C-terminal of paxillin consists of four tandem LIM domains, which mediate protein-protein interactions. Also, its N-terminal contains several proline-rich SH3 binding motifs. Also, it has many serine/threonine and tyrosine phosphorylation sites, which are important intermediate components of cell signaling transduction. The multiple domains of paxillin can bind to a series of signaling proteins and structural proteins, and mediate cell-signaling transduction. It has been found that paxillin plays important roles in cell adhesion and migration. Previous studies⁷ showed that paxillin can serve as a downstream signal of the FAK/Src signaling pathway and be phosphorylated. The paxillin phosphorylation sites at tyrosines 31 and 118 can bind to Crk SH2 domain, which promotes the formation of Crk-DOCK180 complex and, thus, activate the Rac pathway to enhance lamellipodia extension and increase cell mobility. Also, Crk SH3 domain can bind to C3G (a Ras guanine nucleotide exchanging factor) to activate Ras pathway^{8,9}. Paxillin tyrosines 31 and 118 can also bind to the SH2 domain of p120 Ras GAP to separate p190 RhoGAP from itself. Free p190 RhoGAP can inhibit RhoA in the focal adhesion and, thus, promote Rac-mediated lamellipodia extension and enhance cell migration^{10,11}. The binding of Src and the paxillin phosphorylation

site tyrosine 118 can induce the change of paxillin structure and lead to the binding of ERK, which causes the accumulation of a large amount of ERK proteins at the focal adhesion¹². Also, paxillin can bind to Raf and MEK at the focal adhesion to activate the ERK pathway, which can enhance cell diffusion and mobility¹³. After the binding of ARF-GAP/PKL complex with paxillin LD4 domain, PIX and PAK are successively activated to trigger Cdc42/Rac activities at the focal adhesion, which can inhibit Rho expression and promote directional cell and lamellipodia extension^{14,15}. Paxillin contains multiple phosphorylation sites and structural domains and, thus, can bind to the above signal proteins and structural proteins, which makes it a “transfer station” of signaling transduction.

LD Domain

LD domain is located at the N-terminal of paxillin and regulates the signal transduction of paxillin. Its binding site to FAK and Vinculin is highly conserved¹⁶, with five leucine and aspartic acid-binding motifs (LD1-LD5) and the common sequence LD \times LL \times XL. It is considered as the binding platform for FAK and Vinculin⁷. Although these motifs are similar, they have different protein-protein interactions. LD1 mediates the interactions to Actopaxin¹⁷ (it is located at the focal adhesion and serves as the binding protein of paxillin and Actin), Integrin-linked kinase (ILK), Vinculin and HPV E6 protein. LD2 binds to FAK, Vinculin and the proline-rich Tyrosine kinase 2. LD4 binds to Actopaxin¹⁸. LD3 has been degenerated and lacks the conserved sequence. Paxillin kinase linker may bind to focal adhesion kinase 3, Clathrin and polyA-binding protein¹⁹. So far, although the phosphorylation of FAK tyrosine in embryonic stem cells needs LD5, the proteins that can bind to the degenerated LD3 domain have not been found yet²⁰. Paxillin LD4 domain is a particularly important site for regulating Rho GTPase signals.

LIM Domain

The C-terminal of paxillin has four tandem domains, and each domain consists of a double zinc-finger motif and includes two cysteine- and histidine-rich sequences. This special structure is initially found in the homeodomain proteins Lin-11, Isl-1 and Mec-3. Therefore, it is called the LIM domain²¹. The two zinc finger structures of LIM domain are bundled together by hydrophobic interaction. Every zinc finger consists of

two antiparallel β -sheets, which are separated by a turn and have a short α -helix at the terminal. The third LIM domain is the critical structure for paxillin to bind with focal adhesion proteins. LIM domain not only mediates the localization of paxillin on the focal adhesion and actin cytoskeletons, but also mediates the interactions to many proteins including PTP-PEST, tubulin, glucocorticoid receptors, and androgen, etc. Also, there are synergic actions between the adjacent LIM domains which jointly regulate the subcellular localization, and protein binding. Moreover, there are many phosphorylation sites distributing in the whole paxillin protein, such as those of tyrosine and serine/threonine, etc. These phosphorylation sites are critical for mediating cell-signaling transduction. Paxillin shows tyrosine phosphorylation under the stimulation by cytokines and growth factors to generate an SH2-binding domain, which is an important way in regulating protein-protein interaction²⁰. Paxillin shows serine/threonine phosphorylation when induced by Interleukin-3 (IL-3), muscular contraction, virus infection, etc. This process is important for cell localization and adhesion.

Biological Functions of Paxillin

Paxillin is one of the proteins that was firstly discovered to be related to cell adhesion. Paxillin and Integrins constitute a key-site of the focal adhesion between the cell and extracellular matrix. As an intracellular adaptor protein, paxillin can bind to a series of signaling proteins and structural proteins, such as tubulin, actin, vinculin, and actopaxin. These proteins are essential for embryonic development, damage repair, tumor metastasis-related cell migration. Paxillin is not only involved in focal adhesion assembly, but also plays important roles in cell morphological change, movement, adhesion, and signaling transduction. The abnormal expression of paxillin has a correlation with the occurrence, invasion, and metastasis of tumors²².

Paxillin Regulates Cell Morphology and Movement

Previous experimental studies found that the normal expression of paxillin was necessary for normal cell proliferation, differentiation, adhesion, and signaling transmission. Paxillin expression is related to the changes in cell morphology and mobility. Under low or no paxillin expression, cell morphology turns round, the formation of filamentous lamellipodia is affected, and the

cell adhesion and mobility in culture medium are decreased²³. Paxillin-deficient fibroblasts can impair cell migration, which indicates that paxillin can regulate cell dissemination and mobility²⁴. The assembly and disassembly of focal adhesion regulate cell adhesion and movement, thus affecting tumor metastasis. These processes are also regulated by paxillin²⁵. Paxillin tyrosine phosphorylation-regulated cell movement is a hot research field. The role of paxillin tyrosine phosphorylation in cell migration was firstly verified during studying the movement of NBTII bladder cancer cells. Petit et al⁹ discovered that paxillin tyrosine phosphorylation was closely related to cell migration and the binding to CrkII. The paxillin mutations tyr31F/tyr118F stop cell migration, which can be rescued by overexpressing wild-type paxillin or CrkII. The coupling of paxillin and CrkII is essential to the transformation of epithelial mesenchymal cells²⁶. In MDCK cells, overexpression of CrkII induces the localization of paxillin at the focal adhesion and stimulates the dissociation of lamellipodia from the cells. It has been speculated that paxillin tyrosine phosphorylation mediated by Integrins and growth-factor receptors stimulates the formation of paxillin-Crk complex at local focal adhesion. Crk-DOCK180 then activates Rac, leading to the extension of lamellipodia and accelerates cell migration. Interestingly, tyr118 prefers cell adhesion targets, while tyr31 prefers the stimulation of growth factor²⁷. Some studies discovered that serine phosphorylation can also regulate cell movement²⁸; after the mutation of serine-phosphorylation site, cell mobility is limited.

Paxillin Participates in Cell Signaling Transduction

As a signaling protein, paxillin is involved in Integrin-mediated signaling transduction. Integrins are an important class of cell surface molecules. They mediate the adhesion between cells as well as between cells and extracellular matrix. They are involved in multiple physiological processes, such as cell growth, development, differentiation, and apoptosis. After the activation of Integrins and the corresponding ligands, multiple FAK proteins are recruited to the focal adhesion to make tyrosine phosphorylated and FAK activated. paxillin may also participate in this process; activated FAK can bind to Src SH2 domain, which induces the mutual activation of these two tyrosine kinases and leads to a further signaling transduction. Paxillin is a substrate of

FAK and Src. After phosphorylation, paxillin generates certain SH2 domain-binding sites, and Crk is the main adapter protein for these binding sites. Crk SH3 domain can bind to C3G, which is considered as a Ras guanine nucleotide exchange factor. Therefore, paxillin tyrosine phosphorylation can activate the Ras pathway through Crk. It has been verified that paxillin can be directly constrained at the cytoplasmic tail of $\alpha 4$ -Integrin receptor, and paxillin phosphorylation is a critical step of Integrin signaling transduction.

Neovascularization

Previous researches have shown that angiogenesis needs endothelial cells, hematopoietic cells, pericytes and smooth muscle cells. The essence of angiogenesis is the proliferation, migration, and recombination of endothelial cells²⁹. During early embryonic development, the development of vessel trees provides the embryo with oxygen and nutrients³⁰. In E7.5 mouse embryo, mesoderm cells outside the yolk sac are gathered into clusters and indicate the formation of blood island and the initial stage of hemoglobin accumulation³¹. Soon after this stage, the blood island differentiate between the outer layer of endothelial cells and the nucleus of hemocytes³². Meanwhile, the embryonic stem cells proximal to the lateral mesoderm gather and assemble the cardiac tubes connecting with the anterior intestinal portal³³. Angioblasts form a pair of dorsal aorta; then, they are gathered at the centerline to form a single tube. The allantoic mesoderm cells generate umbilical vessels. The allantois in extraembryonic coelom undergoes a rapid development and is integrated with the chorionic extra-embryonic mesoderm³¹. This integration releases a start signal, the differentiation of chorionic vessels, which indirectly connects to the maternal placental vascular system. These early activities are called angiogenesis, which mean that blood-vessel precursor cells are regenerated into blood vessels. With the embryonic development, vascular trees grow and sprout. Vascular endothelial cells are disintegrated and reconstructed into blood vessels. Endothelial progenitor cells can directly be differentiated into vascular endothelial cells³⁴. Therefore, they are also called angioblasts. In 1997, Asahara et al³⁵ firstly applied immunomagnetic beads to isolate CD34+ cells with endothelial-cell-like morphology from peripheral blood. They are named as EPCs³⁶. Functional EPCs express three markers, CD133 (initially called AC133, a transmembrane polypeptide consisting of 865 amino acids, with

the molecular weight of 120 kD, derived from human bone marrow and fetal liver³⁷, and expressed in hemopoietic stem cells and progenitor cells), CD34 and vascular endothelial growth factor receptor-2 (VEGFR-2, also called KDR)^{38,39}. Different from mature vascular endothelial cells, early progenitor cells can simultaneously express multiple antigens, such as CD133, CD34, and KDR, but not E-cadherin and blood coagulation factor VIII (vWF). The EPCs with these features, mainly distribute in the bone marrow, have a high proliferative capacity and share the common origin (hemangioblasts) with hemopoietic stem cells. Hemangioblasts are bi-potential stem cells with CD133, CD34 and fetal liver kinase-1 (FLK-1) phenotypes. During embryonic development, hemangioblasts can differentiate into hemopoietic stem cells and EPCs. EPCs then differentiate into vascular endothelial cells. Endothelial cells undergo extension, interconnection, migration and separation, and form a tube-like structure. At this time, VEGF receptor will be activated. These cells interact with intercellular and extracellular matrix to provide relevant positional information⁴⁰. The vascular endothelial growth factor was firstly named and purified by Leung et al⁴¹ from the *in vitro* culture solution of bovine pituitary follicular stellate cells in 1989. It is a growth factor specifically functioning in vascular endothelial cells⁴². VEGF is a member of the platelet-derived growth factor family. It is the central factor controlling neovascularization and a mitogen specific to vascular endothelial cells. Previous studies⁴³ have shown that VEGF played important roles in the formation and growth of *in situ* tumor and the growth of metastatic tumor. It can promote tumor growth mainly by facilitating neovascularization. Terman et al⁴⁴ transformed the VEGF overexpression vector into the PANCL cells, and injected these cells into the nude mice with low VEGF expression. Compared with the nude mice injected with empty-vector PANCL cells, the nude mice injected with VEGF-overexpression -vector PANCL cells showed a significant larger tumor volume, which indicated that VEGF could promote tumor angiogenesis and tumor growth. Leukemia cells could regulate their survival through the autocrine of inner and outer VEGF/VEGFR-2⁴⁵. Previous studies demonstrated that paxillin and VEGF have mutual interactions. Wu et al⁴⁶ found that paxillin and VEGF had high positive expression rates in hepatocellular carcinoma (HCC) tissues. Zuo et al⁴⁷ reported that paxillin and VEGF showed high expressions

in lung cancer tissue, which was closely related to lymphatic metastasis. Lu et al⁴⁸ found that the VEGF expressed in Kaposi's sarcoma could promote paxillin tyrosine phosphorylation, enhance paxillin expression, and was related to the migration and infiltration of tumor cells²².

Conclusions

Paxillin and VEGF extensively exist in corneal neovascularization tissues and participate in the development, progression and metastasis of new vessels. Both of their expressions are closely related to corneal neovascularization, which indicates that paxillin and VEGF may be the factors affecting corneal neovascularization. Paxillin can regulate the biology functions of HUVECs induced by VEGF-A. The treatment targeting paxillin can inhibit neovascularization and metastasis. Paxillin may become an important target of anti-neovascularization therapies. However, there are still some problems to be solved. For example, the specific roles of paxillin in cell migration are still controversial. The interactions between paxillin and VEGF or other factors are also not very clear. We believe that with the research progress on the molecular mechanisms of angiogenesis, these problems will be gradually solved, and molecular target-based treatment will bring a broad prospect to the clinic treatment of neovascularization.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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