Molecular mechanism of vasculogenic mimicry in tumors

Mechanizm molekularny mimikry naczyniowej w nowotworach

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Pielęgniarstwo Chirurgiczne i Angiologiczne 2015; 1: 22–27

Submitted: 26.02.2014, accepted: 19.01.2015

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Summary

Vasculogenic mimicry (VM) is a new model of tumor angiogenesis, which refers to the de novo formation of perfusable, matrix-rich, vasculogenic-like networks by virtue of the plasticity of aggressive tumor cells and the reconstruction of extracellular matrix (ECM). However, little is known about the molecular underpinnings of this phenomenon. To sum up, interactions of the critical VM-modulating pathways such as vascular (VE-cadherin, EphA2, VEGFR), embryonic and/or stem cell (Nodal, Notch4, CD133+, CD271), and hypoxia-related (HIF-1α, Twist1) signaling pathways are necessary for the formation of vasculogenic mimicry. What is more, accompanied by anti-angiogenic therapy of tumors, the use of VM is becoming more and more important.

Key words: vasculogenic mimicry (VM), molecular mechanism, tumors.

Streszczenie

Mimikra naczyniowa (VM) to nowy model angiogenezy nowotworowej, który dotyczy tworzenia się de novo perfuzyjnych sieci bogatych w macierz, zbliżonych do waskulogennych ze względu na plastyczność agresywnych komórek nowotworowych oraz rekonstrukcję macierzy pozakomórkowej (ECM). Wiedza na temat molekularnych podstaw tego zjawiska jest jednak niewielka. Reasumując – interakcje decydujących kaskad modulujących mimikrę naczyniową, takich jak kaskada naczyniowa (VE-kadheryna, EphA2, VEGFR), embrionalna i/lub komórek macierzystych (Nodal, Notch4, CD133+, CD271) oraz związana z hipoksją (HIF-1α, Twist1), są niezbędne do tworzenia się mimikry naczyniowej. Ponadto wraz z antyangiogenną terapią nowotworów zastosowanie mimikry naczyniowej staje się coraz ważniejsze.

Słowa kluczowe: mimikra naczyniowa, mechanizm molekularny, nowotwory.

Introduction

Tumor vasculogenic mimicry (VM), discovered in 1999, describes the de novo formation of perfusable, matrix-rich, vasculogenic-like networks by aggressive tumor cells in 3-dimensional matrices in vitro, which parallels matrix-rich networks in patients' aggressive tumors. The tumor cells capable of VM share a plastic phenotype and reconstruction of extracellular matrix (ECM). The molecular mechanism of VM is unclear. A large number of studies have contributed to understanding the molecular pathways supporting VM and indicate that endothelial pathways (VE-cadherin, EphA2, VEGFR), embryo or stem cell pathways (Nodal Notch4, CD133+, CD271), and hypoxia-related pathways (HIF-1 alpha, Twist1) play an important role in VM formation [1].

Formation of tumor VM

Phenotypic remodeling of the tumor cell

Phenotypic remodeling includes the reshaping of the genotype and the remodeling of morphological characteristics. In terms of genotype remodeling, Maniotis *et al*. found that 210 different genes are expressed in VM-positive and VM-negative human melanoma cells, including 15 genes associated with endothelial/vascular phenotype [2]. Moreover, the molecular signature of the tumor cell VM phenotype has revealed up-regulated expression of genes associated with embryonic progenitors, endothelial cells, vessel formation, matrix remodeling, and coagulation inhibitors, as well as down-regulation of genes predominantly associated with lineage-specific phenotype markers [3]. The epi-

thelial-mesenchymal transition (EMT) is a remodeling process whereby epithelial cells reduce epithelial characteristics, such as a decrease of cell-cell contact and down-regulation of E-cadherin, while simultaneously acquiring mesenchymal properties including fibroblast-like shape, increased cell motility and up-regulation of mesenchymal markers such as vimentin and cadherin 5 (CDH5) [4]. Moreover, aberrant expression of EMT regulators was found in VM forming cancer cell lines, Twist1 in human hepatocellular carcinoma (HCC) cells [5], and ZEB1 in colorectal carcinoma cells [6]. In addition, hypoxia can enhance VM capacity of HCC cells through increased Twist1 expression [7]. Collectively, these accumulating findings provide supportive evidence that tumor cells capable of VM exhibit a high degree of phenotypic plasticity.

Extracellular matrix reconstitution

The extracellular matrix reconstruction of tumors mainly takes place within cells, and tumor cells interact with the matrix. The change of extracellular matrix components is advantageous to the formation of VM [8]. In the process of extracellular matrix remodeling, abnormal composition of extracellular matrix degradation is the key step. The matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs) and original urokinase type fibrinolytic enzyme activator (uPA) are of great importance. MMPs increase tumor cell surface adhesion protein receptor and laminin adhesion, and make the LN-5γ2 chain split into γ2' and γ2x to destroy the integrity of the extracellular matrix. At the same time, TIMPs block proteolytic activity and promote the matrix changing to a structure that is helpful to the formation of VM [9-11]. uPA is a serine proteolytic enzyme involved in activation of fibrinolytic enzyme. First of all, uPA combines with the urokinase fibrinolytic enzyme activator receptor (uPAR) to promote PLG into fibrinolytic enzyme (PLM), which degrades ECM, basement membrane protein fiber and protein. Secondly, uPAR is not evenly distributed in tumor cell membranes, but is mainly in front of the contact area between ECM and basement membrane. As a result, the uneven distribution of uPAR and high concentration of fibrinolytic enzyme help to orientate degradation of ECM, making it easier for tumor cells to break the bondage of the matrix [12, 13]. Therefore, uPA plays an important role in tumor invasion, metastasis, angiogenesis and VM [14]. In addition, the apoptosis-related protein caspase-3 performs a non-apoptotic function to promote VM formation of melanoma cells. Inhibition of caspase-3 activity by using low-dose z-DEVD-fmk and down regulation of caspase-3 by specific small interfering RNA reduced VM formation of melanoma cells in vitro [15]. Caspase-3-mediated promotion of VM formation may be attributed to the cleavage of matrix metalloproteinase-2.

Molecular mechanisms of VM formation

The plasticity of aggressive tumor cells and the reconstruction of ECM lead to the formation of VM. The molecular basis of this process is supported by the complex cooperation of signaling pathways; in particular, critical VM modulating genes can be categorized into pathways associated with vascular, embryonic and/or stem cell, and hypoxia signaling. Although a myriad of genes having a relationship with VM have been reported, interactions of these three pathways play the role of a platform (Figure 1).

Vascular signaling pathways

The first vascular-associated gene shown to be involved in VM is the VE-cadherin gene (CDH5). In 2006 Hess et al. showed that in VM melanoma VE-cadherin colocalizes with EphA2 at areas of cell-cell contact and that these two molecules are able to directly or indirectly interact during the process of VM [16]. VE-cadherin regulated erythropoietin-producing hepatocellular carcinoma-A2 (EphA2) by mediating it to become phosphorylated through interactions with its membrane-bound ligand ephrin-A1 [17]. Phosphorylated EphA2 subsequently activates phosphoinositide 3-kinase (PI3K), upregulates matrix metalloproteinase (MMP) 14 expression, and activates MMP2. Both MMP14 and MMP2 promote cleavage of the laminin-5γ2 chain into γ2' and γ2x, which in turn stimulate migration, invasion, and VM in melanoma cells [18]. In addition, VE-cadherin expression and activity are enhanced by binding of the transcription factor Twist1 to the VE-cadherin promoter, whereas down-regulation of Twist1 expression leads to decreased VE-cadherin, MMP2, and MMP9 expression and VM formation in human hepatocellular carcinoma cells [5]. Recently, Bcl-2 has also been shown to induce VM in human melanoma cells. Bcl-2 over-expression increased VE-cadherin expression and VM formation under normoxia, whereas Bcl-2 siRNA significantly reduced VE-cadherin expression and VM formation under hypoxia. Therefore, Bcl-2-dependent VE-cadherin over--expression may be an important mechanism by which hypoxia induces VM [19].

In recent years, vascular endothelial growth factor--A (VEGF-A), a well-characterized promoter of endothelial cell proliferation, survival, and angiogenesis, has been linked to VM in both melanoma and ovarian carcinoma. In melanoma, the autocrine secretion of VEGF--A is required for VM, largely through activation of VEGF receptor 1 (VEGFR1). VM appears to be mediated through the activation of PI3K/PKC downstream of VEGFR1, in co-operation with integrin-mediated signaling pathways in melanoma [20-22]. Addition of VEGF-A to ovarian carcinoma cells promoted the up-regulation of VM--associated genes, including the genes for VE-cadherin,

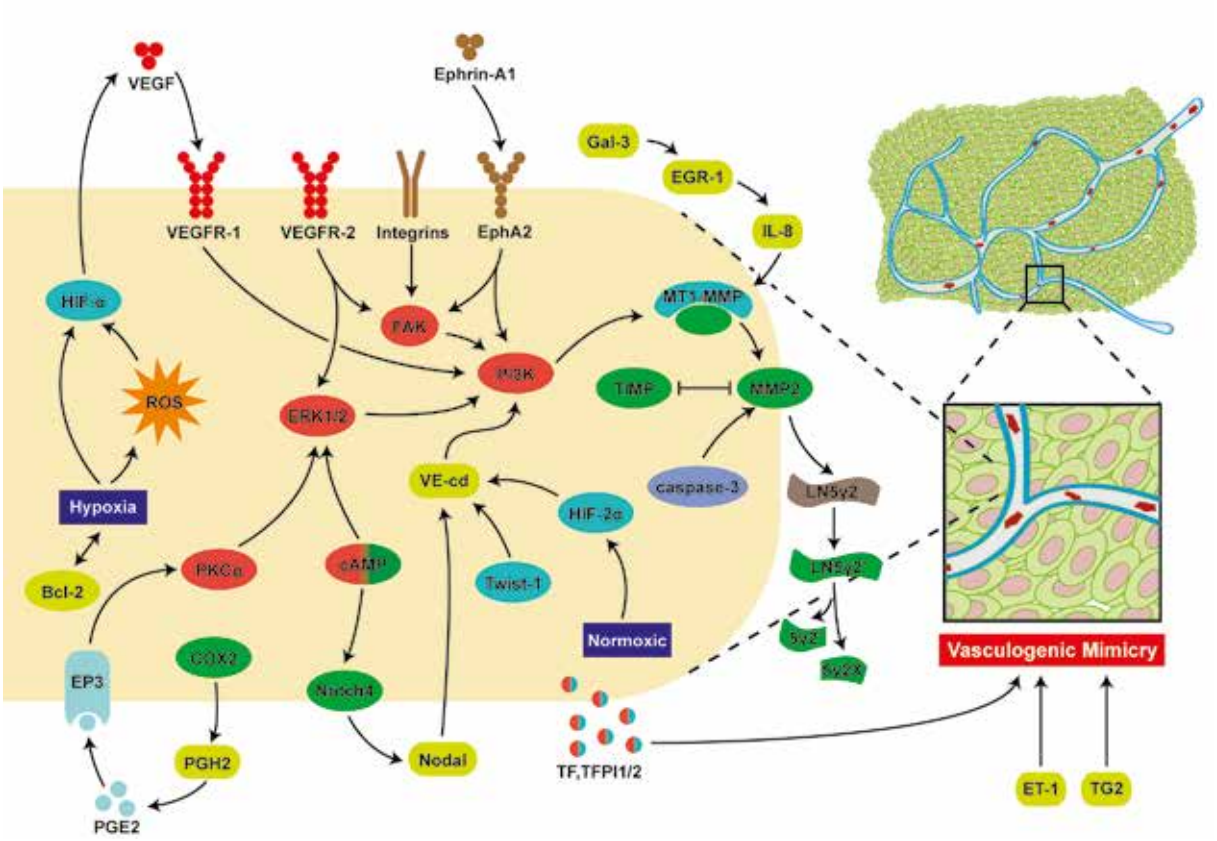

Fig. 1. Pathways associated with vascular, embryonic and/or stem cell, and hypoxia signaling

EPHA2, MMP-2, and MMP-9. Furthermore, it has also been demonstrated that the interaction of EPHA2 and VEGF promotes tumor cell plasticity essential for VM [23]. COX-2, an enzyme responsible for catalyzing the conversion of arachidonic acid into primarily prostaglandin E2 (PGE2), has been found to increase the expression of PGE2, and has also been found to increase the expression of VEGF through a protein kinase C (PKC) mediated pathway. PGE2 binds to a family of prostanoid receptors (prostaglandin E2 receptor subtypes EP1-4), which in turn activate EGF receptor (EGFR) signaling, and PKC-dependent ERK1/2 activation signaling [24]. COX-2/PGE2/EP3 promotion of VM via VEGF has been shown in human pancreatic cancer cells [25]. Recently, it has also been demonstrated that COX-2/EP4 controls tumor growth, angiogenesis, lymphangiogenesis and metastasis of the lungs and lymph nodes in a breast cancer model [26].

VEGF receptor tyrosine kinases (VEGFR1 and VEGFR2) bind VEGF-A in an autocrine or paracrine manner and the activation of the PI3K/Akt pathway, whereas cancer cell invasion and migration have been shown to involve VEGFR1 activation of Src and ERK1/2 pathways [27]. VEGFR1, but not VEGFR2, mediates VEGF-A-induced VM in melanoma cells, and it has been postulated that VM is mediated through the synergistic transduction of VEGF-A/VEGF R1/ PI3K/ PKC and integrin-signaling pathways [22]. However, VEGFR-2 (Flk-1) plays a key role in VM formation of glioblastoma-derived tumor cells via activation of focal adhesion kinase (FAK) and mitogen-activated protein kinase ERK1/2. In contrast, blockade of VEGF activity by the neutralizing antibody bevacizumab fails to recapitulate the impact of VM formation, suggesting that Flk-1-mediated VM is independent of VEGF [28]. However, glioma stem-like cells (GSLCs) preferentially expressed VEGFR-2 that must depend on activating VEGF and then mediates VM [29]. In melanoma cell lines, endothelin-1 (ET-1) in combination with VEGF-C further increased VEGFR-3, MAPK, and AKT phosphorylation and markedly promoted cell migration and vasculogenic mimicry [30]. Although VEGF-C was identified as a lymphangiogenic growth factor and later shown to promote tumor metastasis, the relation with VM may be found soon.

Tissue factor (TF) pathway inhibitor 1 (TFPI-1) and 2 (TFPI-2) are critical genes that initiate and regulate the coagulation pathways. The procoagulant function of TF in highly aggressive melanoma is shown to be regulated by TFPI-1 but not by TFPI-2. Antibody inhibition experiments reveal that TFPI-2 is required for VM in vitro, but plasmin is an unlikely target protease of TFPI-2. Blockade of TFPI-2 suppressed MMP-2 activation, and, therefore, TFPI-2 appears to regulate an essential pathway of VM. Culturing poorly aggressive melanoma

cells on three-dimensional matrix containing recombinant TFPI-2 produces some of the phenotypic change associated with aggressive, vasculogenic melanoma cells. Thus, TFPI-2 contributes to VM plasticity, whereas TFPI-1 has anticoagulant functions of relevance for perfusion of VM channels formed by TF-expressing melanoma cells [31].

Galectin-3 (Gal-3) is a beta-galactoside-binding protein that is involved in cancer progression and metastasis. In vitro, Gal-3 silencing results in loss of tumor cell invasiveness and capacity to form VM. cDNA microarray analysis after Gal-3 silencing revealed that Gal-3 regulates the expression of multiple genes, including VE-cadherin, interleukin-8 (IL-8), fibronectin-1, endothelial differentiation sphingolipid G-protein receptor-1, and MMP-2. Chromatin immunoprecipitation assays and promoter analyses revealed that Gal-3 silencing resulted in a decrease of VE-cadherin and IL-8 promoter activities due to enhanced recruitment of transcription factor early growth response-1 (EGR-1). Moreover, transient over-expression of early growth response-1 in C8161 cells resulted in a loss of VE-cadherin and IL-8 promoter activities and protein expression [32, 33]. Thus, Gal-3 plays an essential role during the acquisition of vasculogenic mimicry and angiogenic properties associated with melanoma progression.

Embryonic and/or stem cell pathways

Nodal signaling pathways are important regulators of human embryonic pluripotency and vertebrate embryonic development [34]. Nodal is a growth factor of the TGFβ superfamily that binds Cripto-1 and activates type I and type II activin-like kinase receptors (ALK4/5/7 and ActRIIB, respectively), which subsequently propagates canonical signaling via Smad2/3 [35, 36]. Activated Smad2/3 regulate the formation of VM by the expression of VE-cadherin. Nodal expression is influenced by Notch, which consists of 4 transmembrane receptors (Notch1-4), whose signaling pathways are critical regulators of vertebrate embryogenesis [36, 37]. Notch signaling is initiated by binding of a Notch ligand, which induces a series of cleavages that generate the release of the Notch intracellular domain (NICD). The NICD translocates to the nucleus and regulates the expression of a number of context-dependent targets, including Nodal [38, 39]. Notch4 functions primarily in vascular development and is enriched in the subpopulation of melanoma cells that form VM [40]. Moreover, in glioblastoma stem-like cells, melanoma cells, triple-negative (TN) breast cancer cells and bladder urothelial carcinoma cells, the stem cell marker CD133+ plays a pivotal role in the formation of VM [41-43]. In addition, one of the most studied tumor stem cell markers, CD271 (also known as nerve growth factor receptor, NGFR or p75NTR), is a neurotrophin receptor, which can bind all of the neurotrophins with similar affinity [44]. It was found that the VM-forming uveal melanoma cell lines in 3D cultures expressed CD271. In contrast, cells grown in 2D cultures and tumor cell subpopulations not participating in VM formation in 3D cultures were negative for CD271 [45]. All the data demonstrate that the embryonic and/or stem cell pathway is very important in VM.

Hypoxia-related signaling pathways

Hypoxia is a hallmark of most solid tumors and can regulate pathways in VM formation. The hypoxia-inducible factor (HIF) complex (composed of HIF-1 and one HIF-subunit: HIF-1, HIF-2, or HIF-3) is a key regulator of oxygen homeostasis in both physiological and pathological environments. Recently, hypoxia has also been shown to induce VM in hepatocellular carcinoma, Ewing's sarcoma, and melanoma [46]. Pertinent to VM, hypoxia can directly modulate VEGF-A, VEGFR1, EPHA2, Twist1, Nodal, osteopontin, and COX-2 gene expression (via HIF/hypoxia response element binding) or indirectly modulate VE-cadherin, TF, and PEDF expression (via activation of an intermediary protein that regulates gene transcription or post-transcriptional protein processing) [47, 48]. In addition, hypoxia can modulate the formation of VM via HIF-1α in human gallbladder carcinoma [49]. Moreover, in the human fibrosarcoma-derived cell line HT1080, the angiogenic marker neuropilin-1 (NRP-1) also plays a vital role in VM formation. Experiments performed with HT1080 cells stably transfected with plasmid constructs expressing shNRP-1 or full-length NRP-1 clearly established that hypoxia-mediated HIF-1α-dependent up-regulation of NRP-1 is a critical molecular event involved in VM [50]. Reactive oxygen species (ROS) regulate VM formation by stabilizing the regulation of HIF-1α and increasing the sensitivity to hypoxia of melanoma cells [51]. Recent studies have shown that pVHL, the protein product of the VHL gene, adjusts the level of HIF-1α to control the expression of VEGF, VE-cadherin, EphA2 and regulation of VM [52, 53].

Anti-tumor research on VM

Although the use of antiangiogenic compounds to target the blood supply of a tumor seems logical, the success of these compounds in the clinic has been very limited. Bevacizumab, sorafenib, and sunitinib target the transdifferentiated endothelial phenotype (VEGF, VEGFRs, PDGFR, RET) with limited efficacy. Clinical use of these drugs has been shown to limit the growth of the primary tumor, but long-lasting effects are rare and typically lead to only moderate benefits for overall survival [54, 55]. There is experimental evidence that spe-

cifically targeting pathways implicated in VM may have success in inhibiting tumor growth. Certainly, a handful of preclinical studies suggest that specific compounds affecting components of the previously described vascular, embryonic, or hypoxia on VM. However, there are only two drugs, the FDA-approved angiogenesis inhibitors thalidomide and rapamycin, that are proved to inhibit VM formation [23]. There are many medicines inhibiting vascular mimicry, which are still in the experimental stage. When in the murine choroidal melanoma model animals were given curcumin once a day at a dose of 100 mg/kg for 18 days, the results indicated that the tumor volume was reduced and the numbers of VM, mosaic vessels, and endothelium-dependent vessels were significantly decreased compared with the control group [56]. That is to say, curcumin has the ability to inhibit the growth of engrafted melanoma VM channels through the regulation of vasculogenic factors that could be related to the down-regulation of the EphA2/PI3K/MMPs signaling pathway. Thus, curcumin has the potential of being a clinical inhibitor of VM of choroidal melanoma [57]. Moreover, the inhibitory effect of nicotinamide on VM formation could be at least partially explained by nicotinamide-driven downregulation of VE-cadherin [58]. More studies have found that not only can lycorine hydrochloride (LH) inhibit the metastatic melanoma cell line C8161 VM structure, but also the VM structure and the volume of transplantation tumor in nude mouse can be inhibited [59]. However, these studies are still at the experimental stage, and the most effective anti-cancer drugs for VM still need more in-depth research.

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