Vasculogenic mimicry, a complex and devious process favoring tumorigenesis – Interest in making it a therapeutic target

Lucas Treps[(1)], Sébastien Faure[(2)], Nicolas Clere[(2)]

(1) Université de Nantes, CNRS, INSERM, CRCINA, F-44000 Nantes, France

(2) Micro et Nanomédecines Translationnelles, MINT, UNIV Angers, UMR INSERM 1066, UMR CNRS 6021, Angers, France

Correspondence :

Nicolas Clere – Pharm D., Ph D

Inserm UMR 1066 CNRS 6021

IBS – CHU Angers

4, rue Larrey

F-49933 Angers Cedex

nicolas.clere@univ-angers.fr

Abstract

Tumor cell vasculogenic mimicry (VM), also dubbed vascular mimicry, describes the plasticity of aggressive cancer cells forming *de novo* vascular networks and is associated with the malignant phenotype and poor clinical outcome. VM is described in a plethora of tumors, including carcinomas, sarcomas, glioblastomas, astrocytomas and melanomas. The presence of VM is associated with a high tumor grade, short survival, invasion and metastasis. A variety of molecular mechanisms and signal pathways participates in VM induction and formation. Due to VM's contribution on tumor progression, more VM-related strategies are being utilized for anticancer treatment. After describing the main features of VM, this review will outline the importance of the tumor microenvironment during this process, and highlight the predominant molecular targets and signaling pathways involved. These data will make it possible to discuss the importance of VM-associated mediators in antitumor therapy and how it could allow to better understand the resistance to anticancer therapy.

Keywords: Cancer cells; tumor microenvironment; vessels; signaling pathways; endothelial cells; anticancer therapy

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Abbreviations

αSMA: alpha smooth muscle actin
CAF: cancer-associated fibroblast
COX2: cyclo-oxygenase 2
CSC: cancer stem cell
Dll: Delta-like ligand
EMT: epithelial-mesenchymal transition
EphA2: ephrin A2 receptor
EVs: extracellular vesicles
FAK: focal adhesion kinase
GSC: glioblastoma stem-like cell
HCC: hepatocellular carcinoma
HDAC: histone deacetylase
HIF: hypoxia-inducible factor
lncRNA: long non-coding RNA
LOXL2: lysyl oxidases like 2
miRNA: micro-RNA
MMP: matrix metalloproteinase
ncRNA: non-coding RNA
NSCLC: non-small cell lung cancer
PAS: periodic acid-Schiff
PGE2: prostaglandin E2
PI3K: phosphatidylinositol-3-kinase
TAM: tumor-associated macrophage
TGF-ß: transforming growth factor beta
TME: tumor microenvironment
uORF: upstream open reading frame
VEGF: vascular endothelial growth factor
VE-PTP: vascular endothelial protein tyrosine phosphatase
VM: vasculogenic mimicry

1. Introduction

Since the studies of Judah Folkman (Folkman, 1971) supplemented by those of Napoleone Ferrara (Kim et al., 1993), tumor growth and metastasis have been explained by a change in the tumor vascular structure through angiogenesis and vasculogenesis. Formation of the tumor vasculature was largely thought to parallel physiological processes of neovascularization, with endothelial sprouting and proliferation being the principal routes of new blood vessel formation. However, this dogma was discussed after the demonstration by Maniotis *et al*. in 1999 (Maniotis et al., 1999) of vascular mimicry (VM) in malignant melanoma. In these tumors, the patterned vascular channels characteristic of aggressive primary and metastatic melanoma are different from angiogenic vessels in that vascular channels in aggressive melanomas are embedded in highly patterned matrix whereas angiogenic vessels are characterized by clusters of vessels and are not patterned. Furthermore, the patterned melanoma vascular channels were not found to be lined by endothelium whereas the contribution of an endothelium to angiogenic vessels is clearly identified. The hypothesis proposed to explain this new vascular architecture was a process of dedifferentiation of tumor cells into an endothelial-like phenotype whose clinical implications are clear: VM-forming tumors were more aggressive than their non-VM counterparts (Qin et al., 2019). Furthermore, VM-lined channels might not respond predictably to conventional anti-angiogenic therapies in melanoma (Schnegg et al., 2015) or other cancers (Angara et al., 2017; Hori et al., 2019).

A major controversial commentary has been proposed on the topic entitled "Vasculogenic mimicry: how convincing, how novel and how significant" (McDonald et al., 2000). Briefly, the main criticisms raised from this commentary focused on different aspects of the original and rather descriptive VM study, which led the authors to the following

questions: (i) Can the VM structures be considered as blood vessels and do they contribute meaningfully to blood flow? (ii) Can the presence or absence of endothelial cells and tumor cells surrounding the vascular lumen be established using unambiguous markers? (iii) Is there an interface between endothelial cells and tumor cells in blood vessel walls? Thus, the relevance of the conclusions from Maniotis *et al*. was questioned, considering the possibility that perhaps VM channels were not functional (Maniotis et al., 1999). Moreover, there were serious technical limitations in identifying VM channels using conventional immunohistochemistry which was prone to artifacts and not entirely objective. Despite these criticisms, it seems that VM has re-entered the spotlight in cancer research with several publications in journal with good scientific impact (Williamson et al., 2016; Xiang et al., 2018) and also with a significant publication rate in the last 10 years (940 hits on *Pubmed* with keywords "vascular mimicry cancer" or "vasculogenic mimicry cancer"). These last studies provide the basis to better define tumor VM which is the source of many resistance to anticancer therapies and also explain the aggressiveness of certain tumors.

2. Characteristics of vascular mimicry

During its vascular stage, tumor nutrition through diffusion is no longer sufficient and expansion of new vasculature is necessary for tumor growth (Folkman, 1990; Gimbrone et al., 1972). Presently, five vascularization modes are now recognized (Carmeliet and Jain, 2011): sprouting and intussusceptive angiogenesis, vessel co-option (Kuczynski et al., 2019), endothelial cell transdifferentiation and VM.

VM is defined as the formation of new blood vessels following the acquisition of vascular cell features or functions by tumor cells of a non-vascular origin. Two distinct VM types have been identified: tubular and patterned matrix VM. Patterned VM is composed of

158 an extracellular matrix of irregular thickness, that is positive for periodic acid-Schiff (PAS),
159 laminin or heparan sulfate, and surrounding islets of cancer cells (Fig. 1A). Although patterned
160 VM networks are shown to conduct fluids, the patterned matrix does not contain a continuous
161 lumen and is morphologically different than blood vessels or their vascular matrix (Folberg
162 and Maniotis, 2004; Maniotis et al., 1999). Furthermore, tubular VM is composed of tumor
163 cells that mimic the normal endothelium to form hollowed and perfused tube-like channels
164 (El Hallani et al., 2010) (Fig. 1B). Whilst blood and lymph vessels are formed by a monolayer
165 of endothelial cells surrounded by a semi-continuous basement membrane, tubules from VM
166 are formed by cancer cells resting on an inner glycoprotein rich matrix (Fig. 2) (Valdivia et al.,
167 2019). In this review, unless specified, the term VM encompasses both tubular and patterned
168 VM types.

169 Ongoing discussions in the field suggest a minimal set of experiments which needs to
170 be performed in order to ascertain the occurrence of VM. Historically, both *in vivo* and *in vitro*
171 models VM structures were positively stained with PAS, and they did not possess endothelial
172 markers such as CD31 or CD34 (Maniotis et al., 1999). Although immunohistology and
173 molecular studies have highlighted the expression of endothelial markers (CD31, CD34, VE-
174 cadherin) in VM structures, $PAS^{+}/CD31^{-}$ staining is currently the most widely used marker for
175 VM. *In vitro*, the molecular characteristics underlying VM are traditionally confirmed in models
176 of highly invasive cell lines able to form tubes on Matrigel®. Finally, the functionality of hollow
177 VM structures has been confirmed *in vitro* upon dye perfusion, and in tumor biopsies with the
178 presence of red blood cells in VM structure's lumen (Ruf et al., 2003; Sood et al., 2001). To
179 date, the occurrence of VM has been shown in various pre-clinical models of breast
180 (Wagenblast et al., 2015), ovarian, prostate (Liu et al., 2012), astrocytoma, glioblastoma,
181 hepatocellular carcinoma (HCC) and lung cancers. Depending on the cancer type, the

incidence of VM positive tumors (based on PAS+CD31- staining) varies considerably (5 to 65%) (Valdivia et al., 2019). However, these investigations need to be taken with a pinch of salt and backed-up by rigorous tissue examination (McDonald et al., 2000).

2.1. Epithelial-mesenchymal transition and VM

VM formation is a sophisticated process involving various mechanisms. While similarities have been shown between epithelial-mesenchymal transition (EMT) and VM, several studies have stressed the main role of tumor microenvironment (TME) during VM.

Initially, EMT has been recognized as a key step during embryonic morphogenesis (Nieto et al., 2016). In different pathophysiological conditions, including cancer, EMT involves various cellular changes in which epithelial cells loosen (i) their attachments to neighboring cells and (ii) their apico-basal cell polarity, while (iii) they start acquiring mesenchymal markers including vimentin, N-cadherin and fibronectin (Thiery, 2002). EMT is highly dynamic, implying transient (dubbed partial-EMT) and reversible states (Ribatti et al., 2020), as well as direct cell-cell interaction or secreted cues (reviewed in (Kim et al., 2020)). EMT implies different signaling pathways including (among other) TGF-β, Wnt, Notch and Hedgehog (Chen et al., 2016; Islam et al., 2016; Ma et al., 2018), as well as the expression of specific transcription factors (Snail, Twist1, SOX4 and ZEB1) acting as repressors of epithelial markers (Gil et al., 2016; Leskela et al., 2019; Ma et al., 2018). This transcriptional remodeling is accompanied by a cadherin switching (Wheelock et al., 2008), particularly from E-cadherin to a panel of mesenchymal cadherins (N-, P-, R-, T- and cadherin 11), that was correlated with tumor progression and invasiveness in squamous cell carcinomas (Miro et al., 2019) and endometrial adenocarcinoma (Fan et al., 2019). Thus, during EMT, cancer cells trade their epithelial characteristics to mesenchymal traits including cytoskeleton reorganization and higher

206 motility (Sannino et al., 2017). Altogether, initiating the first step of the invasion–metastasis
207 cascade, detrimental for patient's prognosis and raising cancer death-toll (Pastushenko and
208 Blanpain, 2019).

209 Interestingly, several studies have shown that EMT is involved in VM formation (Fig. 2,
210 inset i). As proof, it has been reported that ZEB1 small hairpin RNA inhibits both the formation
211 of VM and EMT in triple negative breast cancer (Liang et al., 2018) and colorectal cancer
212 models (Liu et al., 2012). Likewise, it has been found that Twist1 is able to regulate VM
213 formation in astrocytoma, and that its expression is associated with the grade of astrocytoma,
214 one of the most vascularized types tumor in human (Cao et al., 2019; Li et al., 2020). Thus, it
215 suggests a non-negligible role of VM in glioma tumor vascularization. In HCC cell lines,
216 chromatin immunoprecipitation and luciferase assays demonstrated that Twist1 binds the VE-
217 cadherin promoter region and induces VE-cadherin transcription (a molecule which function
218 is developed further below) (Sun et al., 2010). In breast cancer cell lines, Twist1 could mediate
219 VM formation by transcriptional repression of claudin 15 (Zhang et al., 2020). Moreover, in
220 HCC model, it has been confirmed that Twist1 regulates VM formation abilities, through the
221 VE-cadherin/AKT pathway (Sun et al., 2010). Wnt signaling is involved in various physiological
222 processes, such as tumor cell proliferation (Yang et al., 2017), endothelial cell differentiation,
223 and angiogenesis (Shetti et al., 2019). Furthermore, several studies suggested that Wnt
224 signaling induces EMT by repressing glycogen synthase kinase-3β (GSK3β)-mediated
225 phosphorylation that subsequently regulates the phosphorylation/degradation of Snail, an
226 important mediator of EMT (reviewed elsewhere in (Zhong et al., 2020)). Initially, it has been
227 reported that Wnt/β-catenin signaling was involved in VM formation in colon cancer (Qi et al.,
228 2015). Recently, the mechanistic basis of osteosarcoma VM has been extended to 143B and

HOS cell lines where microarray analyzes showed enriched TGF-β and Wnt signaling pathway (Yao et al., 2020).

TGF-β is one of the key EMT driver in various pathologies including cancer (Wendt et al., 2009). Thus, it is not unfounded to assume that TGF-β signaling pathway may also regulate VM. As such, *in vitro* and clinical studies observed that (i) HCC patients with VM and ZEB2 nuclear expression have a shorter survival period than those without expression, and (ii) ZEB2 promotes VM by TGF-β induced EMT (Yang et al., 2015b). Moreover, in a model of SHG44 glioma cells transfected with TGF-β cDNA, EMT regulated by p38/MAPK signaling pathways could participate in VM formation, suggesting a role of this cytokine in the unfavorable evolution of glioma (Ling et al., 2016). As an integral part of the EMT, TGF-β drives the cadherin switching with a loss of E-cadherin and an increase of N-cadherin. Seemingly, VEGF-A plays a similar role during the hypoxia-induced VM in salivary adenoid cystic carcinoma (Wang et al., 2019) and various models of liver tumor (Chen et al., 2019). Thus, these findings suggest that the signaling pathways involved in EMT and VM formation are likely identical or perhaps complementary. A thorough transcriptomic meta-analysis of several VM-competent and TGF-β-treated cancer cell lines could help clarifying this point.

2.2. *Tumor microenvironment, team spirit in VM*

The tumor microenvironment (TME) is the sophisticated cellular milieu in which the tumor develops. Apart from the tumor cells, TME comprises blood and lymphatic vessels, the extracellular matrix and other non-malignant cells including cancer stem cells (CSCs), fibroblasts or immune cells (Wu et al., 2017). Several factors deeply influence the TME and are thus powerful modulators of cell's behaviors. In the following sections, we discuss new findings about how cells from TME contribute to the process of VM (Fig. 2).

Cancer stemness properties and VM

CSCs are rare subpopulations of neoplastic cells within a tumor, usually less than 5%, which are able to generate new tumors in appropriate animal hosts. CSCs have been first identified in human acute myeloid leukemia in 1994, where authors showed that cancer cells grafted into SCID mice produce a large number of $CD34^{+}/CD38^{-}$ subpopulation of leukemic cells (Lapidot et al., 1994). CSCs are also expressed in several solid tumor entities such as breast (Yue et al., 2018), brain (Ren et al., 2018), colon (Lenos et al., 2018), gastric (Zavros, 2017) or prostate (Gorodetska et al., 2019) cancers. Additionally, evidence point out that these CSCs are the cause of tumor initiation, progression, invasion, metastasis, chemoradiotherapy resistance, and relapse underscoring their paramount importance in tumorigenesis (Peitzsch et al., 2017). Among the hypotheses behind the formation of VM, this process would be the ability of VM tumor cells to adopt the pluripotent phenotype of embryonic cells (Seftor et al., 2002). CSC marker expressions associated with VM in human malignant tumors suggest that CSCs may participate in the formation of VM (Fig. 2, inset ii). Different biomarkers are used to characterize and identify CSCs. CD133, also called prominin-1, is a common biomarker of CSCs that was initially described as a biomarker in human hematopoietic stem and progenitor cells (Yin et al., 1997). Currently, CD133 overexpression in various human cancers is considered a significant (unifying?) marker of CSCs (Aghajani et al., 2020; Lathia et al., 2015; Wang et al., 2020b). $CD133^{+}$ tumor cells are able to induce tumor cell proliferation (Pavon et al., 2018), metastasis (Liou, 2019) and to differentiate into other kinds of cells (Virant-Klun et al., 2019; Zhang et al., 2020). Aldehyde dehydrogenase 1 (ALDH1) is another biomarker of CSCs in liver, lung, ovarian, gastric, colorectal or breast cancer (Tomita et al., 2016), and described as a potent inducer of tumor cell proliferation, differentiation and oxidative stress (Vishnubalaji et al., 2018; Zhou et al., 2020). Different studies conducted on various tumor types have shown

277 a correlation between an increase of CD133 and ALDH1 expression, and VM formation. For
278 instance, in osteosarcoma, an aggressive malignant bone tumor in children and adolescents,
279 positive rates of CD133, ALDH1 and VM are significantly higher in tumor tissues compared
280 with control tissues, and positively associated with lymph node and distant metastasis, as well
281 as poor patient overall survival (Bao et al., 2018). In HCC, CD133 and VM are also reported to
282 be significantly higher in tumor tissues than in normal liver tissues (Xu et al., 2018a). Recently,
283 in human and mice triple-negative breast cancer models, it has been confirmed that $CD133^+$
284 and $ALDH1^+$ cells form more VM channels *in vivo* (Sun et al., 2019). These results confirmed
285 CSCs as crucial modulators in the process of VM formation.
286 Because human neural stem cells have been shown to transdifferentiate into
287 endothelial cells (Wurmser et al., 2004), it is established that the differentiation of CSCs into
288 endothelial cells represents one of the hypotheses explaining VM formation. Indeed, renal
289 CSCs can generate endothelial cells *in vitro* and give rise to vessels with a human origin *in vivo*
290 (Bussolati et al., 2008). Furthermore, human breast CSCs undergo endothelial differentiation
291 to generate functional endothelial cells *in vitro* and *in vivo* (Bussolati et al., 2009).
292 Subsequently, these data, obtained from *in vitro* studies, were supplemented by more robust
293 pre-clinical and clinical studies. Thus, in glioblastoma, it has been reported that CSCs generate
294 endothelial cells that form $CD105^+$ (endoglin) tumor vessels (Wang et al., 2010). Moreover,
295 Ricci-Vitiani *et al*. showed that a broad range (20-90%) endothelial cells in glioblastoma
296 harbors the same chromosomal alterations as in tumor cells (Ricci-Vitiani et al., 2010).
297 Although controversial, these studies demonstrate that CSCs in multiple tumors have
298 endothelial differentiation abilities and could contribute to VM formation. This fact is
299 supported by a study conducted on human glioblastoma samples, showing that the so-called
300 glioblastoma stem-like cells (GSCs) may differentiate into $CD31^+/CD34^+$ endothelial cells and

promote VM formation (Mei et al., 2017). In another study conducted on HCC1937/p53 cells, derived from triple-negative breast cancer, cancer cell-transdifferentiated endothelial cells expressed endothelial markers such as VE-cadherin, matrix metalloproteinase (MMP)-2 and -9 and exhibited VM formation ability on Matrigel® (Izawa et al., 2018). Besides, in a mouse embryonic stem cell-induced model of lung CSCs, these cells were able to transdifferentiate into an endothelial cell-like phenotype expressing CD31 and capable of forming tubes on 3D matrix (Prieto-Vila et al., 2016). Taken together, cancer cell's plasticity and transdifferentiation clearly contribute to VM formation. However, presently the exact mechanism driving transdifferentiation and a common underlying signaling across cancer types are lacking but may be worth investigating for therapeutic purpose (see further below).

Hypoxia and VM

Intratumoral hypoxia, which is commonly observed in various solid tumors (Denko, 2008), could trigger numerous signaling pathways which may lead to adverse clinical outcomes including higher cancer invasiveness through promotion of tumor metastasis, EMT or angiogenesis (Chiu et al., 2019) (Fig. 2). Among different hypoxia-related pathways, hypoxia-inducible factors (HIFs) have been studied extensively (Wigerup et al., 2016). As major transcriptional regulators in response to hypoxia, HIFs consist of an oxygen-regulated HIF-α subunit (HIF-1α or HIF-2α) dimerizing with HIF-1β under hypoxia. The dimer then complexes with CREB-cAMP-response element binding protein and activates the transcription of a panoply of target genes harboring hypoxia responsive elements (HRE). Various studies have evaluated the influence of oxygen decrease in the regulation of EMT and VM formation. For instance, Du *et al*. confirmed that hypoxia contributes to VM formation by inducing EMT. Interestingly, HIF-1α expression, analyzed by immunohistochemistry in 71 specimens of

epithelial ovarian cancers, was correlated with loss of E-cadherin expression and up-regulated vimentin expression in 61% of VM-positive patients. Moreover, ovarian cancers with evidence of VM were significantly more likely to have high Twist1, Slug and VE-cadherin expression levels under hypoxia. *In vitro*, ovarian cancer cells presented morphological EMT-like changes under hypoxic conditions (Du et al., 2014). Furthermore, an association of HIF-1α expression and VM formation has been reported in other solid cancers such as melanoma or HCC. An interesting study has confirmed that HIF-1α promotes VM formation in HCC through lysyl oxidases like 2 (LOXL2, a secreted copper-dependent amine oxidase) up-regulation in hypoxic TME. Thus, from clinical HCC tissues, HIF-1α, LOXL2 expression and CD31/PAS double staining were examined by immunohistochemical staining and correlated to poor prognosis. These data have been backed up by series of *in vitro* assays that confirmed a positive relationship between hypoxia, HIF-1α and LOXL2 protein. Thus, HIF-1α was found to induce EMT, HCC cell migration, invasion and VM formation by regulating LOXL2 (Wang et al., 2017). Hence, hypoxia appears as a critical player during EMT and VM formation, and could act on several intricate pathways.

Cancer-associated fibroblasts and VM

Cancer-associated fibroblasts (CAFs) are major components of stromal cells that surround cancer cells, and control proliferation, survival, angiogenesis, metastasis, immunogenicity and resistance to therapies (Su et al., 2018). CAFs constitute the main pool of collagen-producing cells, which directly communicate with cancer cells and various cells from the TME such as endothelial or inflammatory cells. However, the biological properties of CAFs are disparate with different types of CAF driving distinct functional contributions. Besides, previous studies indicated that CAFs arise from different sources of origin including local infiltrating fibroblast,

epithelial or endothelial cells, pericytes or adventitial fibroblasts of the vascular system, or cancer cells themselves undergoing fibroblastic transformation, which may explain CAF's morphological, phenotypical and functional variability (Chen and Song, 2019). In this context, it has been proposed that CAFs could be determinant in VM formation (Fig. 2, inset iii). Thus, in a recent study, VM-competent murine melanoma cells endogenously expressing the matricellular protein CCN2 (also named CTGF), were injected in mice carrying a CAF-specific CCN2 deletion. Interestingly, the absence of fibroblast-derived CCN2 reduced tumor vasculature, including VM occurrence (Hutchenreuther et al., 2018). Furthermore, PAS^+ tissues from human cutaneous melanoma were also positive for the vascular pericyte marker α-smooth muscle actin (αSMA) within the extracellular matrix networks lined by tumor cells. Moreover, when VM-competent tumor cells were co-cultured with pericytes, there was a striking stabilization of the VM networks for up to 96 h (Thijssen et al., 2018). We could parallel these findings with the fact that in glioblastoma, VM structures co-express αSMA and PDGFR, another vascular pericyte marker (Francescone et al., 2012; Scully et al., 2012). The importance of CAFs has also been found in gastric cancers where they promote tumorigenesis through EphA2 (Hong et al., 2018; Nakamura et al., 2005). Pioneer studies in melanoma have previously correlated the activation of the EphA2 receptor tyrosine kinase with VM formation (Hess et al., 2001; Hess et al., 2006) (developed further below). Consequently, EphA2 was significantly associated with VM formation in head and neck squamous cell carcinoma (Wang et al., 2014) and gastric cancer cells (Kim et al., 2019b), which process was lowered in both cases upon EphA2 knockdown. These findings were supplemented by a recent study in gastric cancer AGS cells, showing that VM tube formation was significantly induced by CAF-derived secretome, while conditioned media from normal fibroblasts derived from the same patient had no effect. EphA2 silencing strategy in gastric cancer cells revealed that the CAF-mediated

373 VM promoting effect was mediated through the EphA2-PI3K pathway (Kim et al., 2019a).
374 Altogether, these data suggest that CAFs, as powerful modeler of the TME, may have an
375 underappreciated (yet) function in promoting VM formation.
376
377 *Tumor-associated macrophages and VM*
378 Both innate and adaptive immune cells are present and interact with the tumor cells *via* direct
379 contact or through chemokine and cytokine signaling which shape the behavior of the tumor
380 and its response to therapy. It is well established that tumor-associated macrophages (TAMs)
381 are key regulators in the TME. In solid tumors, the main source of TAM is circulating
382 monocytes rather than proliferating resident macrophages inside tumors. Macrophages can
383 be categorized into M1 and M2 subtypes based on their polarization status. M1 macrophages
384 can be activated by Th1 cytokine interferon gamma and microbial products, while M2
385 macrophages differentiate in response to Th2 cytokines such as IL-4, IL-10 or IL-13 (Qian and
386 Pollard, 2010). In this context of TAMs, M1 macrophages are considered to exert tumoricidal
387 properties whereas M2 macrophages promote tumorigenesis. Both M1 and M2 TAM
388 phenotypes are plastic and reversible, and the TME plays a major role in the regulation of their
389 functional polarization (Duluc et al., 2009; Hagemann et al., 2008). Since inflammatory
390 microenvironment is involved in angiogenesis and plasticity of tumor cells, it has been
391 assumed that inflammatory microenvironment might also promote VM formation (Fig. 2, inset
392 iv). Thus, the infiltration of macrophages in VM-positive areas and the role and underlying
393 mechanism of M2 macrophages in inducing VM formation have been studied in glioblastoma
394 cells (Rong et al., 2016; Zhang et al., 2017). As such, the capability of channel formation in U87
395 cells with or without coculture with M2 (IL-4 stimulated) macrophages has been evaluated.
396 The results revealed that M2 macrophages could enhance the ability to generate vascular

channels in U87 cells under normal oxygen condition. The high αSMA and barely detectable VE-cadherin expression in U87 cells proved that these vascular channels consisted of mural-like cells transdifferentiated from tumor cells. To gain additional insights into the mechanism by which M2 macrophages promote VM formation in U87 cells, the proinflammatory cyclo-oxygenase 2 (COX-2) signaling was investigated in the coculture model. Interestingly, COX-2 expression and the secretion of its downstream effector prostaglandin E2 (PGE2) were up-regulated in U87 cells after M2 macrophages coculture. Hence suggesting that M2 macrophages could induce VM via up-regulating COX-2 expression in glioblastoma cells (Rong et al., 2016). Seemingly, M2-like macrophages enhanced VM through amplification of IL-6 secretion by cancer cells (Zhang et al., 2017). These studies highlight intercellular communications (between M2-like macrophages and glioma cells) that induce VM formation *via* the involvement of different pro-inflammatory mediators.

Within the TME, several factors can influence the outcome and progression of tumorigenesis. For instance, hypoxia and inflammation contribute to cancer cell plasticity, which could reinforce both EMT and VM (Fig. 2). Concomitantly, tumor-associated macrophages are also important mediators of TME by altering tumor inflammatory status, and interacting with several stromal cells. Finally, growing evidences suggest that CAFs and mural cells are likely to contribute and stabilize VM network formation. In depth *in vivo* profiling would be required to properly assess cellular interactions occurring during VM. In particular, we envision that state-of-the-art single cell technologies and ligand-receptor prediction algorithms would allow a thorough understanding of VM, but such studies are currently lacking.

3. Molecular mechanisms in VM

In the following section, we discuss latest insights into the molecular mechanism and signaling pathways driving VM. Non-coding RNA molecules, acting as important epigenetics modifiers, will also be discussed in the context of VM. While a detailed molecular characterization driving VM is still lacking, this section primarily aims at presenting the most acknowledged mechanisms contributing to VM.

3.1. VEGF signaling beyond angiogenesis, involvement in VM

VEGF molecules and its receptors (VEGFRs) are key mediators in physiological (e.g. development and wound healing) and pathological angiogenesis including cancer. VEGF-A, the most studied VEGF member, regulates angiogenesis and vascular permeability by activating VEGFR-1 (FLT-1) and VEGFR-2 (KDR/FLK1), while VEGF-C/-D mostly regulate lymphangiogenesis *via* their receptor VEGFR-3 (FLT-4). In melanoma, the autocrine secretion of VEGF-A is shown to activate PI3K/PKCα and integrin-signaling pathways downstream VEGFR-1, thereby leading to VM (Vartanian et al., 2011). Endothelin 1, through its receptor endothelin-receptor type B (EDNRB), was identified as a melanoma progression marker and is thus associated to an aggressive phenotype (Bittner et al., 2000), and also appeared to play an important role in VM. Indeed, the endothelin 1/EDNRB axis could enhance the expression of VEGFR-3 and its ligands VEGF-C/-D *via* a cross talk involving the activation of c-SRC in a β-arrestin-1-dependent fashion (a pathway also linked to VE-cadherin signaling; see below) (Fig. 3A). This cooperative interaction between EDNRB- and VEGFR-3-mediated signaling resulted in the induction of melanoma cell migration and VM tube formation, and was further inhibited upon the concomitant blockade of these two receptors (Spinella et al., 2013). Peroxiredoxin-2 belongs to an important family of antioxidant proteins. In colorectal cancer, peroxiredoxin-2 expression was associated with VM formation and its knockdown reduced VEGFR-2

activation, allegedly due to an excessive level of oxidative molecules (Zhang et al., 2015a). Similarly, in Epstein-Barr virus-associated malignancies such as nasopharyngeal and gastric cancers where VM was reported, Epstein-Barr virus infection was shown to induce VEGF expression (Xiang et al., 2018; Xu et al., 2018b). However, the exact contribution of VEGF during nasopharyngeal cancer VM is still a matter of debate. Indeed, a first descriptive study indicated that transient silencing of VEGF-A or VEGFR-1 disrupted tubular structures from nasopharyngeal cancer cells, whereas inhibition of VEGFR-2 did not affect the process. Despite the fact LMP-1 and VEGFR-1 expressions were correlated to VM formation, in-depth investigation of the signaling involved was not further investigated by the authors (Xu et al., 2018b). Moreover, further study in the same Epstein-Barr virus-associated cancer outlined a VEGF-independent mechanism (demonstrated by the means of anti-VEGF blocking antibodies and specific anti-VEGFR inhibitors), which activates the PI3K/AKT/mTOR/HIF-1α signaling cascade to induce VM (Xiang et al., 2018).

3.2. CSC-related VEGF signaling in VM

As previously discussed in this review, CSCs are discrete but critical sub-populations influencing tumorigenesis and therapy response. In line with their chemoresistance capacities, CSCs express stemness markers and high levels of drug efflux ATP-binding cassette (ABC) transporters. In melanoma, ABCB5-expressing CSCs co-express Tie-1 and VE-cadherin that are characteristic VM markers in aggressive uveal melanoma cells (Hendrix et al., 2001; Schatton et al., 2008). As compared to $ABCB5^-$ cells, $ABCB5^+$ melanoma cells overexpress VEGFR-1 and preferentially associate with VM structures. As such, VEGFR-1 signaling was important for laminin deposition as VEGFR-1 knockdown xenograft showed reduced VM occurrence and lower tumor growth (Frank et al., 2011). However, melanoma are heterogeneous with some

tumors that may initially respond to anti-VEGF therapy while other are intrinsically resistant. Mirroring this clinical observation, VEGF-A inhibition in anti-VEGF responsive xenografts leads to an increase in VM, CSC markers, as well as the induction of the HIF-1α expression (Schnegg et al., 2015), reported to regulate VM and CSC phenotype in other cancers such as HCC (Ma et al., 2011), Ewing sarcoma (van der Schaft et al., 2005), melanoma (Zhang et al., 2009) and breast cancer (Mao et al., 2020). Examining whether HIF-1α inhibition could prevent the increase in VM induction and CSC maintenance following VEGF-A inhibition in melanoma will be an interesting strategy. Further stressing the importance of cellular context and treatment response heterogeneity, Notch 3 knockdown in melanoma cells exhibiting high endogenous levels of this marker led to retarded tumorigenicity *in vivo* through depleting CSC fraction, corroborated with a reduction in VM. In contrast, Notch 3 silencing affected neither tumor growth nor CSCs in a melanoma cell line with relatively low endogenous Notch 3 expression (Hsu et al., 2017). In several cancers the zinc-finger transcription factor Slug is reported to be an essential mediator of Twist1-induced EMT and metastasis. As such, in HCC Slug is critical to maintain CSC subpopulation, VE-cadherin and mesenchymal markers expression, which further contribute to VM formation (Sun et al., 2013).

CSCs have been particularly well documented in brain tumors, where VM was reported and correlated to higher aggressiveness and shorter overall survival (El Hallani et al., 2010; Liu et al., 2011a; Liu et al., 2011b; Wang et al., 2013; Wang et al., 2012; Yue and Chen, 2005). Immunohistochemistry analysis showed that VM in glioblastoma is principally composed of mural cells co-expressing VEGFR-2, αSMA and PDGFR – the latter two representing vascular pericyte markers – whilst the endothelial marker CD31 was mostly absent (Francescone et al., 2012; Scully et al., 2012). Patient-derived glioblastoma CSCs, the so-called GSCs, were not capable to form any capillary-like structures *in vitro*. Conversely, this ability was retained by

GSC-derived differentiated cells expressing high levels of VEGFR-2 and αSMA, *via* a process dependent on VEGFR-2 and ERK signaling but not of its ligand VEGF (Francescone et al., 2012; Scully et al., 2012). Noteworthy, conflicting reports from another group indicate that VEGF, instead, could stimulate GSC-derived VM, albeit they also describe VEGFR-2 as paramount in inducing VM tube formation (Wu et al., 2017; Yao et al., 2013). These discrepancies could be attributed to the distinct GSC models used: fresh patient-derived *versus* U87 cell line-derived GSCs. Orthotopic mouse tumors with VEGFR-2-silenced GSCs showed decreased tumor growth and $\alpha SMA^{+}CD31^{-}$ vessels without affecting the percentage of mouse $CD31^{+}$ vessels (Scully et al., 2012). Thus, the authors hypothesized that a small population of GSC (<1,5%) could undergo EC transdifferenciation, which participates in tumor angiogenesis as previously described (Ricci-Vitiani et al., 2010; Wang et al., 2010).

3.3. VE-cadherin, more than an endothelial marker

VE-cadherin (also known as CD144 or cadherin-5) is a trans-membrane protein crucial for vascular development, as demonstrated by early lethal phenotype in knockout mouse model (Carmeliet et al., 1999). Endothelial VE-cadherin is a key player controlling cell-cell adhesion and barrier properties (see review in (Treps et al., 2013)). *Via* its extracellular calcium domains, VE-cadherin forms cis- or trans-homodimers to support cell-cell adhesion. Besides, the extracellular calcium domain can bind the endothelial transmembrane receptor-type phosphatase VE-PTP (vascular endothelial protein tyrosine phosphatase) to poise endothelial barrier and keep endothelial permeability at low level (Orsenigo et al., 2012). Of note, VE-cadherin's intracellular domain is the target of numerous post-translational modifications including phosphorylation which orchestrate different signaling cascades depending on the cell type considered (see below).

Although VE-cadherin is an archetypal marker of endothelial cells, a fundamental study reported its expression in highly aggressive human melanoma cells whilst being absent in poorly aggressive melanoma cells derived from the same patient (Hendrix et al., 2001). Highly metastatic melanoma cells had the ability to form vessel-like network in 3D matrix *in vitro*, but this was hampered upon VE-cadherin silencing or CRISPR-Cas9 knockout (Delgado-Bellido et al., 2019; Hendrix et al., 2001). In aggressive melanoma, the molecular pathways downstream VE-cadherin involve its phosphorylation on Y658 residue (pY658) by the focal adhesion kinase (FAK) (Delgado-Bellido et al., 2019) (Fig. 3B). Indeed, FAK silencing or pharmacological inhibition reduced pY658-VE-cadherin levels, even upon VEGF treatment, and decreased VM tube formation. Interestingly, a nuclear pool of pY658-VE-cadherin in complex with p120 was identified in the metastatic uveal cell line MUM2B (Delgado-Bellido et al., 2019). While this phenomenon was already described for p120 and other cadherins such as E-cadherin, this is a premiere for VE-cadherin and would require further validation in other cancer cell types prone to VM. FAK appears to be paramount in controlling the nuclear pY658-VE-cadherin relocalization, which can then associate in complex with Kaiso, a known transcriptional repressor. Upon interaction and binding with p120, Kaiso-transcription repression' activity is counteracted and the target genes induced. Thus, pY658-VE-cadherin could repress the binding of Kaiso to their target genes, induce the expression of CCND1 and Wnt 11, and thus poise the cells to induce VM *in vitro* (Fig. 3B). This recent report highlights how cancer cells such as melanoma cells could highjack existing pathways to induce VM. Recently, a study has developed an original model of culture by coating plates with a fusion protein consisting of the human VE-cadherin extracellular domain coupled to the immunoglobulin G Fc region (Fig. 3B). Strikingly, when the Bel7402 HCC cells were cultured on this substrate, they spontaneously adopted tube-like structures, reminiscent of VM. Concomitant with this

cytoskeleton rearrangement, the cells showed an increased expression in VE-cadherin, EphA2, MMP-2/-9 secretion and PAS staining. Phenotypically, these HCC cells demonstrated an increased migration and invasion, and higher levels of pFAK and pY658-VE-cadherin, associated to several EMT markers (Shuai et al., 2020). Altogether, this would indicate that VE-cadherin has a pivotal place in inducing the EMT, PI3K/MMPs and EphA2/FAK/p-VE-cadherin signaling, and thus trigger VM formation (Fig. 3B).

3.4. VE-PTP, a VE-cadherin-associated receptor

As mentioned above, the transmembrane receptor-type phosphatase VE-PTP associates with VE-cadherin to prevents its pY658 and thus maintains endothelial permeability at minimal level (Orsenigo et al., 2012). Similarly to VE-cadherin, VE-PTP is found expressed in aggressive melanoma cell lines, albeit absent in non-aggressive forms. Despite the fact that VE-PTP knockdown in HUVECs led to an expected increase in pY658-VE-cadherin (due to the absence of VE-PTP phosphatase activity), in aggressive melanoma cells it provoked a distinct outcome with a sharp reduction in pY658- and VE-cadherin total levels. Interestingly enough, immunoprecipitation experiments pointed out that, in this model, VE-PTP does not interact with VE-cadherin but rather with p120 catenin, which is important to prevent VE-cadherin degradation and to sustain the VE-cadherin nuclear pool (Fig. 3B). Noteworthy, the molecular interactions are different in HUVECs where VE-PTP appears linked to VE-cadherin rather than p120 (Delgado-Bellido et al., 2020). Altogether, this highlight that, albeit mimicking normal endothelial cell function, VE-cadherin and VE-PTP behave differently in aggressive melanoma cells to support VM.

3.5. EphA2 signaling in VM

565 The Ephrin family of receptor tyrosine kinases (Eph) and their membrane-tethered ligands
566 (ephrins) constitutes the vastest family of tyrosine kinase receptor. The Ephrin/Eph signaling
567 is involved in various biological processes throughout development, adulthood and
568 pathogenesis (Kania and Klein, 2016). For instance, EphA2, formerly called ECK (epithelial cell
569 kinase), and its ligand ephrin-A1 have been shown to contribute to pathological tumor
570 angiogenesis, albeit *via* an uncharacterized mechanism. Compared to poorly aggressive
571 melanoma cell lines, EphA2 is overexpressed in most advanced melanoma cell lines (up to 14.8
572 fold-change) (Easty et al., 1995; Hess et al., 2007; Hess et al., 2001) and metastatic melanoma
573 biopsies. Furthermore, it is associated with cancer progression and invasion (Easty et al., 1995;
574 Hess et al., 2007). In metastatic aggressive melanoma cell lines, VE-cadherin expression is
575 instrumental in regulating EphA2 localization at cell-cell adhesion complexes, and EphA2
576 tyrosine phosphorylation. Immunostaining and immunoprecipitation indicate that these two
577 transmembrane proteins form a complex *in vitro*, but this might be indirect and could involve
578 additional signaling molecules (Hess et al., 2006). However, even though VE-cadherin-EphA2
579 co-localization has been demonstrated *in situ* in human melanoma sections, a thorough
580 validation is currently lacking (e.g. tube formation). In gastric adenocarcinoma, a positive
581 association between EphA2 expression and VM is highlighted, and further validation studies
582 in gastric cancer cell line indicate that EphA2 knockdown led to a reduction in 3D tube
583 formation *in vitro* (Kim et al., 2019b). Opposingly, EphA2 overexpression in pancreatic
584 adenocarcinoma (Duxbury et al., 2004) and F10-M3 melanoma cells (Parri et al., 2009)
585 increases cell invasiveness in 3D assays, which is in line with the invasive features of VM cells.
586 The F10-M3 metastatic clone of the widely used B16 mouse melanoma cell line has been
587 shown to not express EphA2 and ephrin-A1. However, ectopic expression of EphA2 leads to
588 its tyrosine auto-phosphorylation and downstream signal transduction *via* FAK activation,

589 suggesting a ligand-independent activation (Parri et al., 2009) (Fig. 3B). We can note here that,
590 as mentioned above in melanoma cells, FAK controls pY658-VE-cadherin and its nuclear
591 localization as well as 3D tube formation (Delgado-Bellido et al., 2019). Therefore, although
592 these studies were not performed using the same melanoma cell line, they indicate a possible
593 link between VE-cadherin, EphA2 and FAK signaling in VM.

594 As with many other receptor tyrosine kinases, EphA2 auto-phosphorylation promotes
595 its endocytic internalization, the first step of exosomal sorting (Tkach and Thery, 2016; Walker-
596 Daniels et al., 2002). Such an autocatalytic EphA2 system needs to be poised by the opposing
597 activity of protein tyrosine phosphatases (PTP) and particularly PTP1B located near the
598 pericentriolar recycling endosomes (Nievergall et al., 2010). Another non classical EphA2
599 signaling mechanism was brought to light by studying the secretion profile of doxorubicin-
600 induced senescent cells, and particularly their extracellular vesicle (EV) content. Upon ROS-
601 induced PTP1B inhibition, EphA2 was shed among small EVs and promoted the ERK-
602 dependent proliferation of recipient breast cancer cells through a EphA2/ephrin-A1 reverse
603 signaling (Takasugi et al., 2017). Notwithstanding EphA2 phosphorylation has been linked to
604 VM (Hess et al., 2001; Hess et al., 2006), to our knowledge no study has yet considered the EV
605 communication during VM tube formation (although several studies carried out conditioned
606 media treatments). Additionally, other EphA2 post-translational modifications such as
607 ubiquitination, which balances the EphA2 membranous *versus* internalization remains
608 uncharacterized regarding VM (Sabet et al., 2015).

609

610 *3.6. MMPs, powerful modelers of the tumor microenvironment*

611 These endopeptidases of the zinc-dependent family are implicated in a large variety of
612 physiological processes including wound healing and organogenesis, as well as in pathological

conditions including tumorigenesis. All 28 MMPs are produced as proenzymes, and require a post-translational proteolytic cleavage to generate mature MMPs that have proteolytic activity against a broad range of substrates located on the extracellular matrix. As an example, the substrates from MMP-2/-9 include aggrecan, collagens, elastin, fibronectin, laminins, and glycosaminoglycans. MT1-MMP is a membrane-type collagenase which activates the MMP-2/TIMP-2 complex (Fig. 3A), and is considered as a key mediator of cancer progression, EMT and metastasis (Kessenbrock et al., 2010). Additionally, MMPs play a crucial role in reshaping cell-cell and cell-matrix adhesive characteristics, as well as the extracellular matrix, all processes occurring during VM. As such, first evidence came from the group of Mary J.C. Hendrix with microarray analyses revealing that the expression of several MMPs, MT1-MMP, TIMP-1 and laminin-γ2 were fostered in highly *versus* poorly aggressive patient-derived cutaneous melanoma cell lines (Seftor et al., 2001). Beside, MMP-1/-2/-9, MT1-MMP and laminin-γ2 immunostaining showed a preferential expression in 3D tubular structures formed by aggressive ovarian and melanoma cell lines (Sood et al., 2001) and confirm that MT1-MMP is required for VM tube formation (Fig. 3A).

The Rictor/mTORC2 complex can phosphorylate AKT at Ser473, thereby activating a central component in the PI3K-AKT pathway that regulates the expression of several MMPs. In melanoma cells, Rictor silencing decreased VM channels formation, cell migration and invasion, pSer473-AKT and the expression of MMP-2/-9 (Liang et al., 2017). Myoferlin, a type II membrane protein, is also a regulator of MMP-2 expression and vouch for adequate VM (tube formation and expression of EMT markers) in A375 human cutaneous melanoma cell line (Zhang et al., 2018). Likewise, supporting initial findings in melanoma and ovarian cancers, a recent study in large cell lung cancer cell lines indicates that recombinant MMP-2 could enhance tube formation ability, while recombinant MMP-13 (collagenase-3) or its ectopic

overexpression in H460 and H661 cell lines decreased VM formation (Li et al., 2017a). In melanoma and large cell lung cancer cell lines, the same group showed that while MMP-2 proteolytically cleaves laminin-5 into the 105 kD laminin-5γ2' and 80 kD laminin-5γ2x, MMP-13 can generate small laminin-5 fragments (20 to 40 kD). MMP-13-digested laminin-5 could decrease 3D tube formation but enhanced melanoma cell invasion in Transwell assay (Li et al., 2017a; Zhao et al., 2015). The authors hypothesized that MMP-13 proteolytic action may lead to the cleavage of the laminin-5γ2-containing EGF domain, thereby curbing EGFR downstream Raf/ERK/AKT signaling, as well as the rearrangement of F-actin, vimentin and α-tubulin (Li et al., 2017a). Overall, high MMP-13 expression was inversely correlated with VM occurrence in melanoma patients and H460 subcutaneous tumors, and associated to metastasis in patients (Li et al., 2017a; Zhao et al., 2015). MMP-13 expression was also correlated to poorer overall survival of melanoma patients but this could be attributed to endothelium-dependent tumor vascularization rather than VM (Zigrino et al., 2009).

Fine-tuning histone acetylation pattern by means of histone deacetylases (HDACs) is an important process controlling gene expression profile and frequently deregulated in cancers. This is the case for *HDAC3*, commonly upregulated in solid tumors (Karagianni and Wong, 2007). In glioma, HDAC3 silencing steers to an impaired 3D tube formation along with a decrease in MMP-14, laminin-5γ2 (the latter likely involved in AKT/ERK activation signaling) (Liu et al., 2015). More generally, pharmacological inhibition of class I and II HDACs (HDAC1-10) in glioma cell lines (with SAHA and MC1568), also affects vascular tube formation. The same holds true for GSCs, albeit at higher concentration (2 to 100-fold depending on the HDAC inhibitor), reflecting their well-known resistance to pharmacological treatments (Pastorino et al., 2019). Bromodomain and extra-terminal domain (BET) proteins are epigenetic decoders that recognize acetylated marks on proteins (including histones) and recruit proteins driving

transcriptional activation. JQ1, a BET inhibitor was effective in inhibiting VM of pancreatic ductal adenocarcinoma cells by decreasing the ERK1/2-MMP-2/-9 signaling pathway both *in vitro* and *in vivo* (Zhuo et al., 2019).

A large body of data report the interleukin-33 (IL-33) and its receptor ST2 (encoded by *IL1RL1*) to be important in tumor immune response and cancer cell invasion. As such the IL-33/ST2 axis is now considered as a promising new immunotherapy (Liew et al., 2016). Recently, IL-33 was shown to promote VM tube formation in aggressive melanoma cell lines. Mechanistically, downstream ST2 IL-33 could trigger the ERK1/2 phosphorylation and MMP-2/-9 expression (Yang et al., 2019). However, even though IL-33 was previously described to induce a migrating mesenchymal phenotype, this aspect was not investigated in the context of VM. LOXL2 also plays a key role in extracellular matrix remodeling and particularly in collagen and elastin fibers stabilization which is involved in several processes including cell adhesion, migration, invasion and the EMT. Particularly, LOXL2 interacts and cooperates with Snail to downregulate E-cadherin expression in metastatic carcinoma (Peinado et al., 2005). It is thus not surprising that LOXL2 was found correlated to VM (and to VE-cadherin expression in cancer cells), metastasis and poor patient prognosis in HCC (Shao et al., 2019).

3.7. Semaphorins, potential guides in VM

The Semaphorin family is composed of membrane-anchored and secreted guidance cues which provide a wide repertoire of signals, paramount for neuronal growth, angiogenesis and epithelial barrier integrity (Treps et al., 2013). As such, semaphorins have been linked to EMT in pancreatic (Saxena et al., 2018; Tam et al., 2017), hepatic (Lu et al., 2018; Pan et al., 2016), breast (Yang et al., 2015a), cervical (Song and Li, 2017), ovarian (Tseng et al., 2011), head and neck (Wang et al., 2016) and oral cancers (Liu et al., 2018; Xia et al., 2019). Analogous to the

Ephrin guidance molecules described in the aforementioned section, the expression of the membranous Semaphorin-4D is correlated with VM in a cohort of non-small cell lung cancer (NSCLC). Downstream its receptor plexinB1, Semaphorin-4D triggers the RhoA/ROCK pathway thereby promoting cancer cell migration and invasion, and the occurrence of VM *in vitro* and in xenograft model (Xia et al., 2019). Accordingly, previous studies already reported the involvement of the RhoA/ROCK signaling in VM formation in melanoma, osteosarcoma and HCC (Xia et al., 2017; Xia et al., 2015; Zhang et al., 2014b; Zhang et al., 2015a). Semaphorin-4D silencing also led to a reduction in VE-cadherin, EphA2 and MMP-2/-9, suggesting an intertwined relationship between these partners during VM in NSCLC (Xia et al., 2019). In other models of lung adenocarcinoma, downstream the SAV1-MST1/2-LATS1/2-YAP/TAZ axis Semaphorin-4D was also shown to participate in VM formation (Zhao et al., 2020). Moreover, at the surface of head and neck squamous cell carcinoma cell lines, MT1-MMP proteolytically cleaves Semaphorin-4D and induces Semaphorin-4D-dependent pro-angiogenic response in surrounding endothelial cells (Basile et al., 2006). It is thus not farfetched to hypothesize that this phenomenon could also occur between cancer cells, allowing a directional migration during VM network formation.

3.8. Notch signaling in VM

The Notch pathway is a highly conserved intercellular signaling. Activated by the interaction of the trans-membrane ligands Delta-like (Dll1-4) and Jagged-1/-2 with Notch receptors (Notch 1-4) expressed on adjacent cells, signal transduction is propagated *via* the γ-secretase-driven proteolytic cleavage of Notch receptor intracellular domain (NICD) that is released and form a nuclear transcriptional activator complex (Bray, 2016). Initial work indicates that Notch 1/2, Jagged-1/-2 and Dll1 are upregulated in 'dysplastic nevi' and melanomas as compared to

709 common melanocytic nevi (Massi et al., 2006). Similarly, Notch 4 and Dll4 are overexpressed
710 in aggressive melanoma cell lines where Notch 4 is enriched in melanoma VM networks
711 (Demou and Hendrix, 2008), while Notch 4 blocking antibodies impair VM *in vitro* in a Nodal-
712 dependent manner (NICD can bind and drive Nodal gene expression) (Hardy et al., 2010).
713 Remarkably, Nodal was overexpressed in metastatic melanoma tissues, and curbing
714 downstream signaling pathway (*via* an ALK 4/5/7 inhibitor) could abrogate melanoma cell
715 invasion and tube formation capacity (Topczewska et al., 2006). In breast cancer,
716 overexpression and silencing strategies underline that Nodal promotes VM *via* the Smad2/3
717 pathway (Gong et al., 2016). Additionally, Notch 4 and Dll4 were also found to be associated
718 with VM, metastasis and poor prognosis in NSCLC (Wang et al., 2018). In aggressive human
719 melanoma cell lines, disruption of the Notch signaling with the DAPT γ-secretase inhibitor
720 leads to a seemingly more matured and stabilized *in vitro* tubular network, and melanoma
721 xenografted tumors displays larger and more branched VM channels, associated with an
722 increase in MMP-2 and VEGFR-1 expression (Vartanian et al., 2013). Choriocarcinoma, a rare
723 and highly metastatic gestational trophoblastic cancer, is reported to involve VM with
724 syncytiotrophoblasts lining pseudovascular channels (Shih, 2011), a process that is similar to
725 the embryonic microcirculatory networks (Rai and Cross, 2014). Forskolin is an inducer of
726 syncytiolization, and Forskolin treatment in choriocarcinoma cells resulted in high expression
727 of the VM markers MMP-2/-9, EphA2 and VE-cadherin, tube formation, as well as the
728 activation of the Notch 1 signaling pathway. However, invasive ability and VM markers were
729 reversed by DAPT, suggesting a mechanism whereby Notch-signaling down tunes VM in
730 choriocarcinoma (Xue et al., 2020). Notwithstanding the behavior of trophoblasts is similar to
731 aggressive tumors during differentiation and implantation (Rai and Cross, 2014), Notch 1

signaling seems to have an opposing effect in melanoma and NSCLC (Vartanian et al., 2013; Wang et al., 2018), calling for further investigations before therapeutic applications.

3.9. COXs inflammation in VM

COX-2 is an enzyme responsible for catalyzing the conversion of arachidonic acid into prostaglandins such as PGE2, of paramount importance during inflammation. Subsequently, PGE2 binding to the prostaglandin E2 receptor subtypes (EP1-4) activates the EGFR signaling, and PKC-dependent ERK1/2 activation, pathways described to be involved in VM (Fig. 3A). In breast cancer, COX-2 expression correlates directly with aggressiveness and metastasis. As such, *in vitro* VM tube formation is largely impaired by celecoxib treatment, a selective COX-2 inhibitor, but nearly completely rescued when cells are supplemented with prostaglandin E2. Unbiased bulk RNA-sequencing approach was followed to disentangle the underlying regulatory mechanism. Interestingly, genes related to angiogenesis (e.g. MMP-1, fibronectin, LAMC2) and proliferation (STAT1, EGFR, MAPK) were diminished. Protein array further validated a reduction in major angiogenic proteins such as VEGF and TIMP-1/-2, making a parallel between VM and angiogenic pathways (Basu et al., 2006). Treatments with specific E2 receptor antagonists further highlight the involvement of the EP3 and EP4 receptors to mediate the aggressive phenotype of SUM149 and SUM190 inflammatory breast cancer cells, and mediate VM 3D tube formation (Robertson et al., 2010). Of note, in glioblastoma, the presence of VM channel is associated with the expression of COX-2 and MMP-9 (Liu et al., 2011b). In an elegant *in vitro* coculture model of U87 glioma cell line and IL-4-activated M2 macrophages (as a model of TAMs), tube formation and COX-2 expression were increased. Downstream COX-2, the PGE2/EP1/PKC pathway was of prime importance to induce VM

755 formation, and further confirmed *in vivo* with COX2-deficient U87 xenograft model (Rong et
756 al., 2016).
757
758 *3.10. Non-coding RNAs*
759 Non-coding RNAs (ncRNAs) are not translated into proteins but bear a remarkable variety of
760 biological functions such as RNA processing, transcription and translation regulations. ncRNAs
761 can be grouped according to their length with long non-coding RNAs (lncRNAs) exceeding 200
762 nucleotides in length, small non-coding RNAs between 200 and 50 nucleotides, and tiny
763 ncRNAs for the smallest. The latest group includes siRNAs, miRNAs and piRNAs. In this section,
764 we summarized the mechanistic insights of ncRNAs involved during the process of VM (Table
765 1). For ease of reading, the different ncRNAs are mostly discussed and grouped by tumor
766 type.
767 Similar to melanoma where VM was initially described (Maniotis et al., 1999),
768 aggressive but not poorly invasive triple negative breast cancer cells, efficiently undergo
769 matrix-associated VM under hypoxia. Upon transfection with miR-204, VM tube formation
770 was highly impaired. The pleiotropic effect of the miRNA was assessed using ELISA-based
771 phosphorylation antibodies arrays that revealed a reduced expression and phosphorylation
772 levels of 13 proteins involved in PI3K/AKT, RAF1/MAPK, VEGF, and FAK/SRC signaling.
773 Subsequent validation studies using inhibitors confirmed that PI3K-α and c-SRC signaling were
774 a requisite for VM, and impeded by miR-204 (Lozano-Romero et al., 2020; Salinas-Vera et al.,
775 2018). The lncRNA TP73-AS1 was found upregulated in VM positive tissues from triple
776 negative breast cancer (Tao et al., 2018). Twist1, that accelerates tumor cell VM in breast
777 cancer (Zhang et al., 2014a) harbors a potential miR-490-3p binding site. Tao et al. showed
778 that TP73-AS1 inhibition could release the post-translational suppression of Twist1 induced

779 by miR-490-3p, and thus precluded VM formation (Tao et al., 2018). Interestingly, miR-490-
780 3p was also identified to be play a role in clear cell renal cell carcinoma. Particularly, the
781 NR2C2 (nuclear receptor subfamily 2 group C member 2; aka TR4) could repress the miR-490-
782 3p-dependent vimentin inhibition, and thus favor VM, tumor growth and metastasis (Bai et
783 al., 2018). Dimethylarginine dimethylaminohydrolase 1 (DDAH1) is responsible for the
784 conversion of asymmetric dimethylarginine (ADMA) to citrulline. ADMA is an endogenous
785 inhibitor of the nitric oxide synthase (NOS) and plays a key role in angiogenesis (Eelen et al.,
786 2018). In aggressive breast cancer cell lines, DDAH1 overexpression was counteracted by miR-
787 193b which further disrupted VM (Hulin et al., 2017). The miR-124 was previously shown to
788 inhibit EMT and breast cancer metastasis by targeting Slug (Liang et al., 2013). A report
789 indicates that in cervical cancer, miR-124 down-regulates VM, migration and invasion, as a
790 result from its interaction with AmotL1, a member of the angiomotin family that regulates
791 cell growth and motility.

792 A recent study identified 11 miRNAs that are differently expressed by the SKOV3
793 ovarian cancer cell line during tube formation in an hypoxic environment. Among the so-
794 called “hypoxamiRs”, miR-765 was deeply downregulated upon hypoxia, and could decrease
795 the levels of AKT/SRC axis and exert a negative regulation on VEGF-A by specific binding to its
796 3‘UTR (Salinas-Vera et al., 2019). This time without any link to hypoxia, miR-200a expression
797 was significantly lower in VM positive ovarian cancer. *In vitro* works indicate that it miR-200a
798 and miR-27b negatively regulates EphA2 and VE-cadherin expression, respectively, and thus
799 inhibits VM (Liu et al., 2017; Sun et al., 2014).

800 Expression of the lncRNA LINC00312 is found to be higher in lung adenocarcinoma
801 patients with metastasis. Seemingly, mice inoculated with LINC00312-overexpressing
802 adenocarcinoma cells presented more lung metastatic tumor nodules and higher VM. RNA

pull-down assay identifies YBX1 as the sole interacting partner with LINC00312, that allows the increased expression of VE-cadherin, TGF-β, VEGF-A/-C levels putatively *via* AKT activation (Peng et al., 2018). Oncogenic signaling downstream estrogen receptor beta (ERβ) play an important role in NSCLC progression and EMT. Particularly, ERβ activation up-regulates MALAT1 expression and thus prevent miR-145-5p-mediated NEDD9 downregulation. In this setup, the oncogene NEDD9 seems primordial in inducing VM and cell invasion (Yu et al., 2019). MALAT1 expression was also tightly associated with VM in gastric cancer, via the induction of several signaling pathways including VE-cadherin/β-catenin complex, ERK/MMP and FAK/paxillin signaling (Li et al., 2017b). Investigating whether these pathways are also affected during NSCLC-associated VM would be interesting.

It was shown that c-Myc and SOX2 are important to maintain the pool of liver CSCs, and also to induce VM (Sun et al., 2013). As such, when c-Myc and SOX2 were co-transduced in the HCC cell line HepG2, these cells displayed increased migration, invasion and 3D tube formation properties (Zhao et al., 2018). When compared to HepG2 parental cells, unbiased bulk RNA-sequencing identified 748 differentially expressed ncRNAs, among which lncRNA n339260 that was enriched in the c-Myc/SOX2 overexpressing cells. LncRNA n339260 overexpression in HepG2 was able to promote VM (higher VE-cadherin expression) and CSC markers, and VM tube formation. The miR-31-3p, miR-30e-5p, miR-520c-5p, miR-519c-5p, and miR-29b-1-5p were considered to be downstream the lncRNA n339260 (or regulated by the latter) as their expression increased/decreased following lncRNA n339260 upregulation/downregulation respectively (Zhao et al., 2018). In colorectal cancer, SOX2 is also important for maintaining CSC properties. Ectopic overexpression of miR-450a-5p was shown to suppress the expression of SOX2 by targeting its 3' UTR region and hence restrained SOX2-induced CSC properties, angiogenesis, and VM (Chen et al., 2020). Yang *et al*. studied

827 the mechanism of VM formation in the context of HCC tumor cells and CAF communication.
828 While CAF-secreted TGF-ß and SDF1 promote VM formation in tumor cells, miR-101 could
829 attenuate TGF-ß signaling by targeting its receptor TGF-ßR1 and the downstream effector
830 Smad2 in tumor cells, and simultaneously abrogate SDF1 expression in CAFs. miR-101 is thus
831 pivotal to extinguish VM-promoting signaling in two different cell types (Yang et al., 2016).
832 Zhao et al. found that miR-27a-3p expression was inversely associated with Twist1
833 expression. miR-27a-3p appears to mediate the inhibition of EMT and VM by downregulating
834 VE-cadherin (upon binding to its 3'UTR), and miR-27a-3p down-regulation is associated with
835 metastasis (Zhao et al., 2016a). miR-186 also suppresses Twist1 expression in prostate cancer,
836 and hence thwarted EMT, cell motility and tube formation *in vitro* and metastasis in mouse
837 model (Zhao et al., 2016b).

838 So far, glioma is the tumor entity with the greatest variety of miRNAs and lncRNAs
839 described to play a role in VM. miR-Let-7f halted VM by disturbing periostin-induced
840 migration of glioma cells (Xue et al., 2016). The levels of miR-141 (Li et al., 2018a), miR-26b
841 (Wu et al., 2011) were inversely correlated with the grade of glioma, and both miRNAs were
842 shown to directly target EphA2 an important regulator in VM. Recently, the group of Prof.
843 Xue has studied two lncRNAs (LINC00339 and HOXA-AS2) that are upregulated in human
844 glioma and positively correlated to VM. LINC00339 binds and prevents the action of the miR-
845 539-5p, thereby activating the Twist1/MMP-2/MMP-14 signaling pathway to promote VM
846 (Guo et al., 2018) (Fig. 3B). HOXA-AS2 acts via a different pathway, which first involves its
847 binding to miR-373. Downstream EGFR signaling, miR-373 regulates the expression of VE-
848 cadherin, MMP-2/-9 and thus VM (Gao et al., 2018). Stathmin 1 is a member of the
849 microtubule destabilizing protein family that regulates microtubule dynamics during cell-
850 cycle progression. Stathmin 1 is overexpressed in various cancers and its expression is

positively associated with higher grades of human glioma. In glioma cell lines, stathmin 1 appears to be the target of the (central nervous system) tissue specific miR-9 that refrains growth and VM of ectopic xenografts (Song et al., 2013). Interestingly, over-expression of miR-584-3p paralyses hypoxia-induced VM formation in human A172 glioma cells, by antagonizing ROCK1-dependent stress fiber formation (Xu et al., 2017). As mentioned before, the RhoA/ROCK signaling pathway has been described to participate in VM formation in melanoma, osteosarcoma and HCC (Xia et al., 2017; Xia et al., 2015; Zhang et al., 2014a; Zhang et al., 2015b). Recently, three studies from the same group identified several lncRNAs regulating VM in glioma. Important translational regulations occur from the 3' and 5' untranslated regions (UTR) of mRNAs. Particularly, upstream open reading frames (uORFs), commonly found in the 5'UTR of eukaryotic genes, are important in the initiation of translation. uORFs in a gene initiate nonsense-mediated RNA decay (NMD) that promotes transcript degradation. An uORF was identified in the lncRNA ZNRD1-AS1, namely ZNRD1-AS1-144aa-uORF. ZNRD1-AS1 is highly expressed in glioma and promotes VM formation, while 144aa-uORF was down-regulated and VM hindered. Mechanistically, the lncRNA ZNRD1-AS1 binds to miR-499a-5p, which in turn regulates the ELF1/EMI1 axis to hamper VM formation (Wang et al., 2020a). Therefore, via an intricate regulatory process, the lncRNA ZNRD1-AS1 could foster VM in glioma. The human splicing factor zinc finger, Ran-binding domain containing protein 2 (ZRANB2) is a splicing protein that is part of the supraspliceosome and mediates differential splicing of numerous primary transcripts (Yang et al., 2013). The lncRNA SNHG20 is correlated with poor overall survival and tumor-node-metastasis in several cancer types. In glioma cells, ZRANB2 promoted VM formation by increasing SNHG20 stability. As a consequence, SNHG20 was able to promote Forkhead box K1 degradation, thereby alleviating the transcriptional inhibition of VM-related proteins

MMP-1/-9 and VE-cadherin (Li et al., 2019). Hence, TZRANB2 and SNHG20 were powerful inducers of VM formation in glioma. Another recent report studied the regulation of the poly(A) binding protein cytoplasmic 5 (PABPC5), which binds to the 30 end of the poly(A) tail of most eukaryotic mRNAs. Interestingly, overexpression of the lncRNA HCG15 or PABPC5 promoted VM, while upregulation of the transcriptional repressor ZNF331 greatly curbed VM by directly repressing LAMC2 (coding for Laminin-5γ2, important for VM (Seftor et al., 2001; Sood et al., 2001)) and PABPC5 transcription. By targeting the VM-inhibitor ZNF331, the lncRNA HCG15 could thus promote VM formation in glioma (Jing et al., 2020).

In conclusion, these studies highlight the complexity and diversity of VM-related processes regulated by lncRNAs and miRNAs (Table 1). To our knowledge, presently no study reports the direct implication of EVs during VM. However, these EVs are recognized carriers of several ncRNAs and also modulators of extracellular matrix remodeling, a process greatly involved during VM. As a proof-of-concept, using the user-friendly online EVmiRNA database (Liu et al., 2019), we could identified miR-450a-5p and miR-101-3p as expressed in EVs from colon carcinoma (with a preferential enrichment in microvesicles). As such, in-depth investigations of the implication of EVs and EV-carried ncRNAs may certainly warrant interesting findings in VM formation.

4. VM and cancer therapy

4.1. Cancer resistance

For many aggressive tumors, current therapeutic strategies are insufficient. Thus, VM formation could explain the therapeutic resistances observed with cytotoxic or targeted chemotherapies: residual tumor cells may form VM channels, thereby providing oxygen and nutrients, which support cell proliferation and cancer progression. Not only has VM been

reported in different solid tumors, but tumor cell-originated neovascularization including tumor-derived endothelial cell-induced angiogenesis along with VM have been suggested to be involved in the development of resistance to anti-VEGF therapy as frequently observed in glioblastoma. Anti-angiogenic drugs, mostly affecting VEGF-VEGFR pathway, cause dramatic tumor size reduction and are largely used in glioblastoma therapy as adjuvants to control abnormal vasculature. However, the benefits are transient since glioblastoma rapidly show resistance to anti-angiogenic therapies upon prolonged treatment through the activation of alternative vascularization pathways (Angara et al., 2017; Arbab et al., 2015). Moreover, it has been noted that hypoxia associated with anti-angiogenic therapies may induce VM that represents a mechanism whereby glioblastoma can escape anti-angiogenic therapies (Angara et al., 2017). VM is also reported to represent a non-angiogenic pathway in breast-cancer metastasis. In this last tumor, it has been reported that angiogenic primary breast carcinoma can relapse not only as angiogenic, but also as non-angiogenic lung metastases. The authors propose that this non-angiogenic pathway is a novel pathway of cancer progression and such tumors are likely to be resistant to anti-angiogenic treatment. In addition, the role of anti-angiogenic treatment in VM promotion has been confirmed in a study conducted in triple-negative breast cancer cells. Thus, an enhancement of VM-positive cases has been observed in sunitinib (a VEGFR tyrosine kinase inhibitor)-treated cells in comparison with control cells. These effects were explained by an overexpression of HIF-1α, VE-cadherin and Twist1 (Sun et al., 2017). More recently, a study has been conducted with trastuzumab, a drug that target the receptor tyrosine kinase HER2 in breast cancer cells. Several VM markers were upregulated in trastuzumab-treated cells suggesting that HER-2 positive breast cancer cells can exhibit VM in an angiogenic microenvironment after acquiring trastuzumab resistance

(Hori et al., 2019). Hence, VM may be regarded as one of the major causes of the development of resistance to anti-angiogenic therapy in solid tumors.

4.2. Therapeutic targeting of VM

If it is clearly established that VM participates in the therapeutic resistances of many solid tumors, in this regards it constitutes a promising reservoir for developing novel anticancer therapeutics, and an armamentarium of drugs has been investigated (Table 2). Recently, studies have been conducted with histone deacetylase (HDAC) inhibitors both in glioblastoma and in aggressive triple-negative breast cancer. As previously described, altered expression and/or function of HDAC could play an important role in tumor initiation and progression (Li et al., 2018b) and these enzymes are promising therapeutic targets in various cancers (Sun et al., 2018).

Interestingly, *in vitro* and *in vivo* evidences suggested the antitumor activity of HDAC inhibitors by preventing VM in different cancer models. For instance, it has been found that vorinostat, trichostatin A, entinostat (MS275) and MC1568 significantly decrease tube formation by U87MG and GSCs. These results were explained, at least in part, by a significant decrease of migration and invasion of glioma cells upon HDAC treatments (Pastorino et al., 2019). Furthermore, it has been reported that the specific HDAC inhibitor, entinostat, is able to reduce VM structure formations in various triple-negative breast cancer cells. Thus, this study revealed that treatment of MDA-MB-231, LM2-4, BT-549 or MCF-7 with entinostat epigenetically led to the re-expression of the anti-angiogenic genes, serpin family F member 1 (SERPINF1) and thrombospondin 2 (THBS2), as well as the tumor suppressor genes, phosphatase and tensin homolog (PTEN) and p21. Otherwise, it has been found that entinostat downregulated the expression of VEGF-A, and that of the EMT-related genes,

vimentin and β-catenin (Maiti et al., 2019). These two studies offer interesting perspectives for the use of HDAC inhibitors against VM. However, these studies have limited clinical translation as they lack to evaluate the impact of these molecules on VM formation *in vivo*. No pre-clinical or clinical studies have yet been considered with HDAC inhibitors and it is still too early to seriously envision these molecules as potential therapeutic agents against VM formation.

As previously described, CSCs are main actors in VM formation and are also responsible for the low survival rate of patients with aggressive tumors. After chemo- and radiotherapy, only a small proportion of CSCs are capable to induce recurrence. Furthermore, EMT is also involved in the acquisition of CSC properties. Taken together, these findings suggest that a combination of EMT and CSC targeting may be beneficial for anti-VM formation therapy through a decrease of both invasion and metastasis and an improvement of patient's survival rate. It has been found that salinomycin, a potassium ionophore used as an anticoccidial drug, completely suppressed VM. The most prominent property of this drug is that it selectively kills CSCs (Gupta et al., 2009).

Furthermore, because VEGF-A enhances the ability of melanoma cells to form tube-like structures by activating VEGFR-1 and the downstream PKC-α pathway, it has been suggested to target VEGFR-1 to prevent VM (Fig. 3A). Interestingly, an anti-VEGFR-1 monoclonal antibody (D16F7) has shown antitumor activity by inhibiting chemotaxis, extracellular matrix invasion and VEGFR-1^{+} cancer cells VM in melanoma and glioblastoma, two aggressive tumors (Atzori et al., 2017; Graziani et al., 2016). Taken together, these findings highlight tumor type specificities and could explain the limited efficacy of sorafenib and thus suggesting a therapeutic benefit for tyrosine kinase inhibitors targeting preferentially VEGFR-1.

Various other strategies have been under discovery to inhibit VM formation. Because MMP-2- and MT1-MMP-mediated laminin-γ2 cleavage reportedly induces VM formation, anti-MMP-2 or anti-MT1-MMP blocking antibodies treatment has been proposed to abrogate it (Seftor et al., 2001). A similar outcome was achieved when treating 3D culture of ovarian cancer cells at the onset of tube formation with incyclinide (*aka* Metastat), an inhibitor of MMP activity. However, this treatment could not affect an established network, suggesting a role of MMPs in early steps of tube formation rather than for their stability (Sood et al., 2001). A derivative of incyclinide has made it to the clinics with COL-3 (NSC-683551) to treat patients with refractory metastatic cancers (NCT00001683).

In addition, nanostructured functional drug-loaded liposomes, modified with an HIV peptide lipid-derivative conjugate and containing the chemotherapeutics epirubicin and celecoxib were tested for their effect on VM. The added value of this delivery system was to improve pharmacokinetic properties and bio-distribution of such anticancer agents. As such, tail vein injection of drug-loaded liposomes reduced VM expression via the inhibition of VE-cadherin, FAK, EphA2, HIF-1α, and MMP-9 in invasive breast cancer xenografts in nude mice (Ju et al., 2014). Doxycycline has been used in combination with targeted drugs in clinical trials for patients with advanced cancer. Doxycycline is a semi-synthetic tetracycline which can inhibit MMP activation and cell proliferation, as well as interfere with tumor-related protein synthesis in mammalian cells. Doxycycline has a strong inhibitory effect on malignant cells especially NCI-H446 and A549 lung cancer cells (Cao et al., 2013; Ko et al., 2015). Interestingly, doxycycline treatment prolonged the mouse survival time and partly suppressed the growth of engrafted HCC tumor cells, with an inhibition rate of 43.39% (Meng et al., 2014). In HCC and lung cancer models, doxycycline is capable to inhibit E-cadherin degradation and down-regulates the expression of vimentin protein (Cao et al., 2013; Ko et al., 2015; Meng et al.,

994 2014). These findings show that doxycycline acts upstream of EMT-related signal transduction
995 to inhibit a wide range of cellular function. As such, investigations in HCC revealed that (i) *in*
996 *vitro* doxycycline promoted cell adhesion but hampered HCC cell viability, proliferation,
997 migration and invasion; and (ii) *in vivo* higher amounts of VM and endothelium-dependent
998 vessels were found in the control group than the treatment group (Meng et al., 2014).
999 Among the new molecules used in antitumor therapies, encouraging results have been
1000 obtained with galunisertib (LY2157299), a selective ATP-mimetic inhibitor of TGF-βR1, that
1001 improved glioma prognosis. Thus, from *in vitro* and pre-clinical studies, it has been found that
1002 VM was inhibited by galunisertib through a decrease in VE-cadherin and αSMA expression, as
1003 well as down-regulation of AKT and VEGFR-2 phosphorylation in galunisertib-treated glioma
1004 cells (Zhang et al., 2016).
1005 Recently, several studies involving molecules extracted from plants have been tested
1006 in different tumor models. The properties of brucine, a traditional medicinal herb extracted
1007 from seeds of *Strychnos nux-vomica* have been tested in a triple-negative breast cancer cell
1008 line MDA-MB-231. The authors concluded that brucine inhibited VM through repressing
1009 EphA2 and MMP-2/-9 expression (Xu et al., 2019). Another study evaluated the effects of
1010 polyphyllin I, the main component of *Rhizoma paridis* in VM in HCC. It has been reported that
1011 polyphyllin I blocked VM through an inhibition of PI3K/AKT – Twist1 – VE-cadherin axis
1012 through a regulation of the transcriptional activity of Twist1 (Xiao et al., 2018). In a B16F10
1013 metastatic melanoma model, Bhattacharyya *et al*. proposed lupeol as a substitute for
1014 dacarbazine at the onset of therapeutic resistance because it is able to block VM through a
1015 downregulation of the CSC marker CD133 (Bhattacharyya et al., 2019). If the abovementioned
1016 studies seem exciting for their authors, they remain extremely preliminary and do not
1017 constitute (yet) alternatives to recognized therapies. Indeed, no precise pharmacological data

has been demonstrated for the different molecules tested. The works do not specify the pharmacodynamic or pharmacokinetic characteristics of these molecules. For instance, brucine is weakly liposoluble (Li and Wang, 2017) and hardly crosses the barrier formed by the plasma and nuclear membranes. Also, if the pharmacological targets are intracellular, its availability may be limited. In addition, in the case of lupeol, the doses used to block VM are very high (40 mg/kg) and the published data did not provide clear evidence of a true antitumor activity of this molecule. According to our previous work (Pautu et al., 2019), the mice model B16F10 is extremely aggressive and, for us, it is not possible to think that lupeol is sufficient by itself to limit the progression of this metastatic melanoma.

5. Conclusion

As highlighted above, a plethora of signaling pathways and multiple growth factors and their receptors are described to promote VM. Therefore, one could predict that hindering a single signaling pathway by means of an inhibitor would have a limited suppressive effect on VM, since other signaling pathways would immediately compensate and eventually restore the process of switching to VM phenotype. In fact, inhibitors such as galunisertib, LY294002 (a PI3K inhibitor), doxycycline, everolimus or regorafenib showed only limited efficacy. For several months, it has been recognized that therapeutic combinations are essential to treat aggressive cancers. Therefore, it is essential to increase the number of studies combining different therapeutic approaches in order to limit tumor development by blocking significantly VM formation.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Figure legends

Table 1. Non-coding RNAs involved during vasculogenic mimicry

TNBC: triple negative breast cancer; HCC: Hepatocellular carcinoma.

Table 2. Summary of therapeutics targeting vasculogenic mimicry

CSCs: Cancer stem cells; GSCs: Glioblastoma stem cells; HCC: Hepatocellular carcinoma.

Fig. 1. The two types of vascular mimicry.

A) Patterned vasculogenic mimicry (VM) forms architectural patterns of loops and networks as revealed by periodic acid-Schiff (PAS)-stained phase contrast micrograph of metastatic cutaneous melanoma C8161 3D culture. Pictures acquire after 1 week on Matrigel; magnification x40. **B)** Tubular VM showing melanoma cancer cells that lined a vascular channel containing red blood cells, but without endothelial cells identified. Hematoxylin-eosin-staining; magnification x100. Reprinted from "Vascular Channel Formation by Human Melanoma Cells in Vivo and in Vitro: Vasculogenic Mimicry" by Maniotis et al., The American Journal of Pathology, 155/3, 739-752, 1999, with permission from Elsevier.

Fig. 2. Partners in crime during vasculogenic mimicry in tumors.

Tumor vascularization can be achieved by several processes such as the classical endothelium-dependent sprouting angiogenesis vasculogenic (bottom right), and endothelium-independent tubular vasculogenic mimicry (VM). Such blood conducting network is built by cancer cells themselves resting on an extracellular matrix (ECM) rich in laminin, stained by periodic acid Schiff (PAS; depicted as the red outline) without endothelial markers such as CD31 or CD34. In the tumor microenvironment, various cell types foster VM network formation. (i) The epithelial-mesenchymal transition (EMT) whereby cancer cells lose their polarity and acquire a migratory mesenchymal phenotype upon several signaling pathways (TGF-ß, Wnt, Notch, etc.) and transcription factors (Snail, Twist1, ZEB1/2). (ii) Cancer associated fibroblasts (CAFs) are important player in ECM remodeling and their secretome contribute to VM formation. (iii) Tumor associated macrophages (TAMs) contribute to cancer cell transdifferentiation and pro-inflammatory cytokines. (iv) Cancer stem cells (CSCs) harbor pluripotent features allowing them to differentiate and form VM network, but also to do endothelial transdifferentiation contributing to tumor angiogenesis.

Fig. 3. Molecular highlights involved during vasculogenic mimicry formation.

A) VEGF-A, downstream the activation of VEGFR-1, lead to the activation of the PI3K/PKC pathway, converging to AKT with actin reorganization, and thus promoting vasculogenic mimicry (VM). COX-2 transforms the arachidonic acid into prostaglandin E2 (PGE2) that acts on its receptor E2 to foster the PKC/ERK1/2 axis, important during VM. Endothelin, via its receptor EDNRB, leads to ß-arrestin-mediated c-SRC activation, which in turn cooperates with VEGF-C/-D to phosphorylate VEGFR-3. The VEGFR-3 activation leads to MMP2 transcription into its pro-MMP-2 form that require proteolytic activation from the MT1-MMP/TIMP2 complex. The active MMP-2 can then trigger the cleavage of laminin-ɣ2 into laminin-5ɣ2’,

which stimulates VM. **B)** Downstream VEGF-1/VEGFR-2 or EphA2 receptors, VE-cadherin can be phosphorylated on its tyrosine 658 (Y658) by FAK. Downstream PI3K/AKT and ERK activation, the expression of several MMPs (MMP-14/-2/-9) is induced and will activate the MMP-2-mediated VM functions in a similar manner as described for MT1-MMP. Via pY658, FAK mediates VE-cadherin nucleus translocation and the VE-PTP/p120 complex appears important to control this process. In the nucleus, upon binding to p120, the transcription repressor Kaiso can no longer repress the VM-inducible genes CCND1 and WNT11. When cancer cells are cultured on a matrix coated with the extracellular domain of VE-cadherin, the EphA2 and PI3K/AKT pathways are activated, and VM formation initiated.

References

Aghajani, M., Mokhtarzadeh, A., Aghebati-Maleki, L., Mansoori, B., Mohammadi, A., Safaei, S., Asadzadeh, Z., Hajiasgharzadeh, K., Khaze Shahgoli, V., and Baradaran, B. (2020). CD133 suppression increases the sensitivity of prostate cancer cells to paclitaxel. Mol Biol Rep *47*, 3691-3703.

Angara, K., Rashid, M. H., Shankar, A., Ara, R., Iskander, A., Borin, T. F., Jain, M., Achyut, B. R., and Arbab, A. S. (2017). Vascular mimicry in glioblastoma following anti-angiogenic and anti-20-HETE therapies. Histol Histopathol *32*, 917-928.

Arbab, A. S., Jain, M., and Achyut, B. R. (2015). Vascular Mimicry: The Next Big Glioblastoma Target. Biochem Physiol *4*.

Atzori, M. G., Tentori, L., Ruffini, F., Ceci, C., Lisi, L., Bonanno, E., Scimeca, M., Eskilsson, E., Daubon, T., Miletic, H.*, et al.* (2017). The anti-vascular endothelial growth factor receptor-1 monoclonal antibody D16F7 inhibits invasiveness of human glioblastoma and glioblastoma stem cells. Journal of experimental & clinical cancer research : CR *36*, 106.

Bai, J., Yeh, S., Qiu, X., Hu, L., Zeng, J., Cai, Y., Zuo, L., Li, G., Yang, G., and Chang, C. (2018). TR4 nuclear receptor promotes clear cell renal cell carcinoma (ccRCC) vasculogenic mimicry (VM) formation and metastasis via altering the miR490-3p/vimentin signals. Oncogene *37*, 5901-5912.

Bao, Z., Cheng, Z., and Chai, D. (2018). The expressions of CD133, ALDH1, and vasculogenic mimicry in osteosarcoma and their clinical significance. Int J Clin Exp Pathol *11*, 3656-3663.

Basile, J. R., Castilho, R. M., Williams, V. P., and Gutkind, J. S. (2006). Semaphorin 4D provides a link between axon guidance processes and tumor-induced angiogenesis. Proc Natl Acad Sci U S A *103*, 9017-9022.

Basu, G. D., Liang, W. S., Stephan, D. A., Wegener, L. T., Conley, C. R., Pockaj, B. A., and Mukherjee, P. (2006). A novel role for cyclooxygenase-2 in regulating vascular channel formation by human breast cancer cells. Breast Cancer Res *8*, R69.

Bhattacharyya, S., Mitra, D., Ray, S., Biswas, N., Banerjee, S., Majumder, B., Mustafi, S. M., and Murmu, N. (2019). Reversing effect of Lupeol on vasculogenic mimicry in murine melanoma progression. Microvasc Res *121*, 52-62.

Bittner, M., Meltzer, P., Chen, Y., Jiang, Y., Seftor, E., Hendrix, M., Radmacher, M., Simon, R., Yakhini, Z., Ben-Dor, A.*, et al.* (2000). Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature *406*, 536-540.

Bray, S. J. (2016). Notch signalling in context. Nat Rev Mol Cell Biol *17*, 722-735.

Bussolati, B., Bruno, S., Grange, C., Ferrando, U., and Camussi, G. (2008). Identification of a tumor-initiating stem cell population in human renal carcinomas. FASEB J *22*, 3696-3705.

Bussolati, B., Grange, C., Sapino, A., and Camussi, G. (2009). Endothelial cell differentiation of human breast tumour stem/progenitor cells. J Cell Mol Med *13*, 309-319.

Cao, J., Song, Y., Bi, N., Shen, J., Liu, W., Fan, J., Sun, G., Tong, T., He, J., Shi, Y.*, et al.* (2013). DNA methylation-mediated repression of miR-886-3p predicts poor outcome of human small cell lung cancer. Cancer Res *73*, 3326-3335.

Cao, W., Xu, C., Li, X., and Yang, X. (2019). Twist1 promotes astrocytoma development by stimulating vasculogenic mimicry. Oncol Lett *18*, 846-855.

Carmeliet, P., and Jain, R. K. (2011). Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nat Rev Drug Discov *10*, 417-427.

Carmeliet, P., Lampugnani, M. G., Moons, L., Breviario, F., Compernolle, V., Bono, F., Balconi, G., Spagnuolo, R., Oosthuyse, B., Dewerchin, M.*, et al.* (1999). Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. Cell *98*, 147-157.

Chen, J., Chen, S., Zhuo, L., Zhu, Y., and Zheng, H. (2020). Regulation of cancer stem cell properties, angiogenesis, and vasculogenic mimicry by miR-450a-5p/SOX2 axis in colorectal cancer. Cell Death Dis *11*, 173.

Chen, K. J., Li, Q., Wen, C. M., Duan, Z. X., Zhang, J. Y., Xu, C., and Wang, J. M. (2016). Bleomycin (BLM) Induces Epithelial-to-Mesenchymal Transition in Cultured A549 Cells via the TGF-β/Smad Signaling Pathway. J Cancer *7*, 1557-1564.

Chen, Q., Lin, W., Yin, Z., Zou, Y., Liang, S., Ruan, S., Chen, P., Li, S., Shu, Q., Cheng, B., and Ling, C. (2019). Melittin Inhibits Hypoxia-Induced Vasculogenic Mimicry Formation and Epithelial-Mesenchymal Transition through Suppression of HIF-1α/Akt Pathway in Liver Cancer. Evid Based Complement Alternat Med *2019*, 9602935.

Chen, X., and Song, E. (2019). Turning foes to friends: targeting cancer-associated fibroblasts. Nat Rev Drug Discov *18*, 99-115.

Chiu, D. K., Zhang, M. S., Tse, A. P., and Wong, C. C. (2019). Assessment of Stabilization and Activity of the HIFs Important for Hypoxia-Induced Signalling in Cancer Cells. Methods Mol Biol *1928*, 77-99.

Delgado-Bellido, D., Bueno-Galera, C., López-Jiménez, L., Garcia-Diaz, A., and Oliver, F. J. (2020). Endothelial Phosphatase VE-PTP Participates in Vasculogenic Mimicry by Preventing Autophagic Degradation of VE-Cadherin. Front Oncol *10*, 18.

Delgado-Bellido, D., Fernández-Cortés, M., Rodríguez, M. I., Serrano-Sáenz, S., Carracedo, A., Garcia-Diaz, A., and Oliver, F. J. (2019). VE-cadherin promotes vasculogenic mimicry by modulating kaiso-dependent gene expression. Cell Death Differ *26*, 348-361.

Demou, Z. N., and Hendrix, M. J. (2008). Microgenomics profile the endogenous angiogenic phenotype in subpopulations of aggressive melanoma. J Cell Biochem *105*, 562-573.

Denko, N. C. (2008). Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat Rev Cancer *8*, 705-713.

Du, J., Sun, B., Zhao, X., Gu, Q., Dong, X., Mo, J., Sun, T., Wang, J., Sun, R., and Liu, Y. (2014). Hypoxia promotes vasculogenic mimicry formation by inducing epithelial-mesenchymal transition in ovarian carcinoma. Gynecol Oncol *133*, 575-583.
Duluc, D., Corvaisier, M., Blanchard, S., Catala, L., Descamps, P., Gamelin, E., Ponsoda, S., Delneste, Y., Hebbar, M., and Jeannin, P. (2009). Interferon-gamma reverses the immunosuppressive and protumoral properties and prevents the generation of human tumor-associated macrophages. Int J Cancer *125*, 367-373.
Duxbury, M. S., Ito, H., Zinner, M. J., Ashley, S. W., and Whang, E. E. (2004). EphA2: a determinant of malignant cellular behavior and a potential therapeutic target in pancreatic adenocarcinoma. Oncogene *23*, 1448-1456.
Easty, D. J., Guthrie, B. A., Maung, K., Farr, C. J., Lindberg, R. A., Toso, R. J., Herlyn, M., and Bennett, D. C. (1995). Protein B61 as a new growth factor: expression of B61 and up-regulation of its receptor epithelial cell kinase during melanoma progression. Cancer Res *55*, 2528-2532.
Eelen, G., de Zeeuw, P., Treps, L., Harjes, U., Wong, B. W., and Carmeliet, P. (2018). Endothelial Cell Metabolism. Physiol Rev *98*, 3-58.
El Hallani, S., Boisselier, B., Peglion, F., Rousseau, A., Colin, C., Idbaih, A., Marie, Y., Mokhtari, K., Thomas, J. L., Eichmann, A.*, et al.* (2010). A new alternative mechanism in glioblastoma vascularization: tubular vasculogenic mimicry. Brain *133*, 973-982.
Fan, M. J., Liang, S. M., He, P. J., Zhao, X. B., Li, M. J., and Geng, F. (2019). Dusp6 inhibits epithelial-mesenchymal transition in endometrial adenocarcinoma via ERK signaling pathway. Radiol Oncol *53*, 307-315.
Folberg, R., and Maniotis, A. J. (2004). Vasculogenic mimicry. APMIS *112*, 508-525.
Folkman, J. (1971). Tumor angiogenesis: therapeutic implications. N Engl J Med *285*, 1182-1186.
Folkman, J. (1990). What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst *82*, 4-6.
Francescone, R., Scully, S., Bentley, B., Yan, W., Taylor, S. L., Oh, D., Moral, L., and Shao, R. (2012). Glioblastoma-derived tumor cells induce vasculogenic mimicry through Flk-1 protein activation. J Biol Chem *287*, 24821-24831.
Frank, N. Y., Schatton, T., Kim, S., Zhan, Q., Wilson, B. J., Ma, J., Saab, K. R., Osherov, V., Widlund, H. R., Gasser, M.*, et al.* (2011). VEGFR-1 expressed by malignant melanoma-initiating cells is required for tumor growth. Cancer Res *71*, 1474-1485.
Gao, Y., Yu, H., Liu, Y., Liu, X., Zheng, J., Ma, J., Gong, W., Chen, J., Zhao, L., Tian, Y., and Xue, Y. (2018). Long Non-Coding RNA HOXA-AS2 Regulates Malignant Glioma Behaviors and Vasculogenic Mimicry Formation via the MiR-373/EGFR Axis. Cell Physiol Biochem *45*, 131-147.
Gil, D., Ciołczyk-Wierzbicka, D., Dulińska-Litewka, J., and Laidler, P. (2016). Integrin-linked kinase regulates cadherin switch in bladder cancer. Tumour Biol *37*, 15185-15191.
Gimbrone, M. A., Leapman, S. B., Cotran, R. S., and Folkman, J. (1972). Tumor dormancy in vivo by prevention of neovascularization. J Exp Med *136*, 261-276.
Gong, W., Sun, B., Zhao, X., Zhang, D., Sun, J., Liu, T., Gu, Q., Dong, X., Liu, F., Wang, Y.*, et al.* (2016). Nodal signaling promotes vasculogenic mimicry formation in breast cancer via the Smad2/3 pathway. Oncotarget *7*, 70152-70167.
Gorodetska, I., Lukiyanchuk, V., Peitzsch, C., Kozeretska, I., and Dubrovska, A. (2019). BRCA1 and EZH2 cooperate in regulation of prostate cancer stem cell phenotype. Int J Cancer *145*, 2974-2985.

Graziani, G., Ruffini, F., Tentori, L., Scimeca, M., Dorio, A. S., Atzori, M. G., Failla, C. M., Morea, V., Bonanno, E., D'Atri, S., and Lacal, P. M. (2016). Antitumor activity of a novel anti-vascular endothelial growth factor receptor-1 monoclonal antibody that does not interfere with ligand binding. Oncotarget *7*, 72868-72885.
Guo, J., Cai, H., Liu, X., Zheng, J., Liu, Y., Gong, W., Chen, J., Xi, Z., and Xue, Y. (2018). Long Non-coding RNA LINC00339 Stimulates Glioma Vasculogenic Mimicry Formation by Regulating the miR-539-5p/TWIST1/MMPs Axis. Mol Ther Nucleic Acids *10*, 170-186.
Gupta, P. B., Onder, T. T., Jiang, G., Tao, K., Kuperwasser, C., Weinberg, R. A., and Lander, E. S. (2009). Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell *138*, 645-659.
Hagemann, T., Lawrence, T., McNeish, I., Charles, K. A., Kulbe, H., Thompson, R. G., Robinson, S. C., and Balkwill, F. R. (2008). "Re-educating" tumor-associated macrophages by targeting NF-kappaB. J Exp Med *205*, 1261-1268.
Hardy, K. M., Kirschmann, D. A., Seftor, E. A., Margaryan, N. V., Postovit, L. M., Strizzi, L., and Hendrix, M. J. (2010). Regulation of the embryonic morphogen Nodal by Notch4 facilitates manifestation of the aggressive melanoma phenotype. Cancer Res *70*, 10340-10350.
Hendrix, M. J., Seftor, E. A., Meltzer, P. S., Gardner, L. M., Hess, A. R., Kirschmann, D. A., Schatteman, G. C., and Seftor, R. E. (2001). Expression and functional significance of VE-cadherin in aggressive human melanoma cells: role in vasculogenic mimicry. Proc Natl Acad Sci U S A *98*, 8018-8023.
Hess, A. R., Margaryan, N. V., Seftor, E. A., and Hendrix, M. J. (2007). Deciphering the signaling events that promote melanoma tumor cell vasculogenic mimicry and their link to embryonic vasculogenesis: role of the Eph receptors. Dev Dyn *236*, 3283-3296.
Hess, A. R., Seftor, E. A., Gardner, L. M., Carles-Kinch, K., Schneider, G. B., Seftor, R. E., Kinch, M. S., and Hendrix, M. J. (2001). Molecular regulation of tumor cell vasculogenic mimicry by tyrosine phosphorylation: role of epithelial cell kinase (Eck/EphA2). Cancer Res *61*, 3250-3255.
Hess, A. R., Seftor, E. A., Gruman, L. M., Kinch, M. S., Seftor, R. E., and Hendrix, M. J. (2006). VE-cadherin regulates EphA2 in aggressive melanoma cells through a novel signaling pathway: implications for vasculogenic mimicry. Cancer Biol Ther *5*, 228-233.
Hong, H. N., Won, Y. J., Shim, J. H., Kim, H. J., Han, S. H., Kim, B. S., and Kim, H. S. (2018). Cancer-associated fibroblasts promote gastric tumorigenesis through EphA2 activation in a ligand-independent manner. J Cancer Res Clin Oncol *144*, 1649-1663.
Hori, A., Shimoda, M., Naoi, Y., Kagara, N., Tanei, T., Miyake, T., Shimazu, K., Kim, S. J., and Noguchi, S. (2019). Vasculogenic mimicry is associated with trastuzumab resistance of HER2-positive breast cancer. Breast Cancer Res *21*, 88.
Hsu, M. Y., Yang, M. H., Schnegg, C. I., Hwang, S., Ryu, B., and Alani, R. M. (2017). Notch3 signaling-mediated melanoma-endothelial crosstalk regulates melanoma stem-like cell homeostasis and niche morphogenesis. Lab Invest *97*, 725-736.
Hulin, J. A., Tommasi, S., Elliot, D., Hu, D. G., Lewis, B. C., and Mangoni, A. A. (2017). MiR-193b regulates breast cancer cell migration and vasculogenic mimicry by targeting dimethylarginine dimethylaminohydrolase 1. Sci Rep *7*, 13996.
Hutchenreuther, J., Vincent, K., Norley, C., Racanelli, M., Gruber, S. B., Johnson, T. M., Fullen, D. R., Raskin, L., Perbal, B., Holdsworth, D. W*., et al.* (2018). Activation of cancer-associated fibroblasts is required for tumor neovascularization in a murine model of melanoma. Matrix Biol *74*, 52-61.
Islam, S. S., Mokhtari, R. B., Noman, A. S., Uddin, M., Rahman, M. Z., Azadi, M. A., Zlotta, A., van der Kwast, T., Yeger, H., and Farhat, W. A. (2016). Sonic hedgehog (Shh) signaling

promotes tumorigenicity and stemness via activation of epithelial-to-mesenchymal transition (EMT) in bladder cancer. Mol Carcinog *55*, 537-551.
Izawa, Y., Kashii-Magaribuchi, K., Yoshida, K., Nosaka, M., Tsuji, N., Yamamoto, A., Kuroyanagi, K., Tono, K., Tanihata, M., Imanishi, M*., et al.* (2018). Stem-like Human Breast Cancer Cells Initiate Vasculogenic Mimicry on Matrigel. Acta Histochem Cytochem *51*, 173-183.
Jing, F., Ruan, X., Liu, X., Yang, C., Wang, D., Zheng, J., Xue, Y., Shen, S., Shao, L., Yang, Y*., et al.* (2020). The PABPC5/HCG15/ZNF331 Feedback Loop Regulates Vasculogenic Mimicry of Glioma via STAU1-Mediated mRNA Decay. Mol Ther Oncolytics *17*, 216-231.
Ju, R. J., Li, X. T., Shi, J. F., Li, X. Y., Sun, M. G., Zeng, F., Zhou, J., Liu, L., Zhang, C. X., Zhao, W. Y., and Lu, W. L. (2014). Liposomes, modified with PTD(HIV-1) peptide, containing epirubicin and celecoxib, to target vasculogenic mimicry channels in invasive breast cancer. Biomaterials *35*, 7610-7621.
Kania, A., and Klein, R. (2016). Mechanisms of ephrin-Eph signalling in development, physiology and disease. Nat Rev Mol Cell Biol *17*, 240-256.
Karagianni, P., and Wong, J. (2007). HDAC3: taking the SMRT-N-CoRrect road to repression. Oncogene *26*, 5439-5449.
Kessenbrock, K., Plaks, V., and Werb, Z. (2010). Matrix metalloproteinases: regulators of the tumor microenvironment. Cell *141*, 52-67.
Kim, H., Lee, S., Shin, E., Seong, K. M., Jin, Y. W., Youn, H., and Youn, B. (2020). The Emerging Roles of Exosomes as EMT Regulators in Cancer. Cells *9*.
Kim, H. S., Won, Y. J., Shim, J. H., Kim, H. J., Kim, B. S., and Hong, H. N. (2019a). Role of EphA2-PI3K signaling in vasculogenic mimicry induced by cancer-associated fibroblasts in gastric cancer cells. Oncol Lett *18*, 3031-3038.
Kim, H. S., Won, Y. J., Shim, J. H., Kim, H. J., Kim, J., Hong, H. N., and Kim, B. S. (2019b). Morphological characteristics of vasculogenic mimicry and its correlation with EphA2 expression in gastric adenocarcinoma. Sci Rep *9*, 3414.
Kim, K. J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H. S., and Ferrara, N. (1993). Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature *362*, 841-844.
Ko, H., Jeon, H., Lee, D., Choi, H. K., Kang, K. S., and Choi, K. C. (2015). Sanguiin H6 suppresses TGF-β induction of the epithelial-mesenchymal transition and inhibits migration and invasion in A549 lung cancer. Bioorg Med Chem Lett *25*, 5508-5513.
Kuczynski, E. A., Vermeulen, P. B., Pezzella, F., Kerbel, R. S., and Reynolds, A. R. (2019). Vessel co-option in cancer. Nat Rev Clin Oncol *16*, 469-493.
Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., Paterson, B., Caligiuri, M. A., and Dick, J. E. (1994). A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature *367*, 645-648.
Lathia, J. D., Mack, S. C., Mulkearns-Hubert, E. E., Valentim, C. L., and Rich, J. N. (2015). Cancer stem cells in glioblastoma. Genes Dev *29*, 1203-1217.
Lenos, K. J., Miedema, D. M., Lodestijn, S. C., Nijman, L. E., van den Bosch, T., Romero Ros, X., Lourenço, F. C., Lecca, M. C., van der Heijden, M., van Neerven, S. M*., et al.* (2018). Stem cell functionality is microenvironmentally defined during tumour expansion and therapy response in colon cancer. Nat Cell Biol *20*, 1193-1202.
Leskela, S., Pérez-Mies, B., Rosa-Rosa, J. M., Cristobal, E., Biscuola, M., Palacios-Berraquero, M. L., Ong, S., Matias-Guiu Guia, X., and Palacios, J. (2019). Molecular Basis of Tumor Heterogeneity in Endometrial Carcinosarcoma. Cancers (Basel) *11*.

Li, C., Chen, Y., Zhang, Q., Guo, C., Chen, F., Xi, S., Zeng, J., Ke, C., Sharma, H. S., and Chen, Z. (2020). Expression of Twist associated to microcirculation patterns of human glioma correlated with progression and survival of the patient. Int Rev Neurobiol *151*, 201-217.
Li, G., Huang, M., Cai, Y., Ke, Y., Yang, Y., and Sun, X. (2018a). miR-141 inhibits glioma vasculogenic mimicry by controlling EphA2 expression. Mol Med Rep *18*, 1395-1404.
Li, S., and Wang, X. P. (2017). In vitro and in vivo evaluation of novel NGR-modified liposomes containing brucine. Int J Nanomedicine *12*, 5797-5804.
Li, T., Zhang, C., Hassan, S., Liu, X., Song, F., Chen, K., Zhang, W., and Yang, J. (2018b). Histone deacetylase 6 in cancer. J Hematol Oncol *11*, 111.
Li, X., Xue, Y., Liu, X., Zheng, J., Shen, S., Yang, C., Chen, J., Li, Z., Liu, L., Ma, J*., et al.* (2019). ZRANB2/SNHG20/FOXK1 Axis regulates Vasculogenic mimicry formation in glioma. J Exp Clin Cancer Res *38*, 68.
Li, Y., Sun, B., Zhao, X., Wang, X., Zhang, D., Gu, Q., and Liu, T. (2017a). MMP-2 and MMP-13 affect vasculogenic mimicry formation in large cell lung cancer. J Cell Mol Med *21*, 3741-3751.
Li, Y., Wu, Z., Yuan, J., Sun, L., Lin, L., Huang, N., Bin, J., Liao, Y., and Liao, W. (2017b). Long non-coding RNA MALAT1 promotes gastric cancer tumorigenicity and metastasis by regulating vasculogenic mimicry and angiogenesis. Cancer Lett *395*, 31-44.
Liang, W., Song, S., Xu, Y., Li, H., and Liu, H. (2018). Knockdown of ZEB1 suppressed the formation of vasculogenic mimicry and epithelial-mesenchymal transition in the human breast cancer cell line MDA-MB-231. Mol Med Rep *17*, 6711-6716.
Liang, X., Sun, R., Zhao, X., Zhang, Y., Gu, Q., Dong, X., Zhang, D., Sun, J., and Sun, B. (2017). Rictor regulates the vasculogenic mimicry of melanoma via the AKT-MMP-2/9 pathway. J Cell Mol Med *21*, 3579-3591.
Liang, Y. J., Wang, Q. Y., Zhou, C. X., Yin, Q. Q., He, M., Yu, X. T., Cao, D. X., Chen, G. Q., He, J. R., and Zhao, Q. (2013). MiR-124 targets Slug to regulate epithelial-mesenchymal transition and metastasis of breast cancer. Carcinogenesis *34*, 713-722.
Liew, F. Y., Girard, J. P., and Turnquist, H. R. (2016). Interleukin-33 in health and disease. Nat Rev Immunol *16*, 676-689.
Ling, G., Ji, Q., Ye, W., Ma, D., and Wang, Y. (2016). Epithelial-mesenchymal transition regulated by p38/MAPK signaling pathways participates in vasculogenic mimicry formation in SHG44 cells transfected with TGF-β cDNA loaded lentivirus in vitro and in vivo. Int J Oncol *49*, 2387-2398.
Liou, G. Y. (2019). CD133 as a regulator of cancer metastasis through the cancer stem cells. Int J Biochem Cell Biol *106*, 1-7.
Liu, R., Yang, K., Meng, C., Zhang, Z., and Xu, Y. (2012). Vasculogenic mimicry is a marker of poor prognosis in prostate cancer. Cancer Biol Ther *13*, 527-533.
Liu, T., Zhang, Q., Zhang, J., Li, C., Miao, Y. R., Lei, Q., Li, Q., and Guo, A. Y. (2019). EVmiRNA: a database of miRNA profiling in extracellular vesicles. Nucleic Acids Res *47*, D89-D93.
Liu, T. J., Guo, J. L., Wang, H. K., and Xu, X. (2018). Semaphorin-7A contributes to growth, migration and invasion of oral tongue squamous cell carcinoma through TGF-β-mediated EMT signaling pathway. Eur Rev Med Pharmacol Sci *22*, 1035-1043.
Liu, W., Lv, C., Zhang, B., Zhou, Q., and Cao, Z. (2017). MicroRNA-27b functions as a new inhibitor of ovarian cancer-mediated vasculogenic mimicry through suppression of. RNA *23*, 1019-1027.
Liu, X., Wang, J. H., Li, S., Li, L. L., Huang, M., Zhang, Y. H., Liu, Y., Yang, Y. T., Ding, R., and Ke, Y. Q. (2015). Histone deacetylase 3 expression correlates with vasculogenic mimicry through

the phosphoinositide3-kinase / ERK-MMP-laminin5γ2 signaling pathway. Cancer Sci *106*, 857-866.
Liu, X. M., Zhang, Q. P., Mu, Y. G., Zhang, X. H., Sai, K., Pang, J. C., Ng, H. K., and Chen, Z. P. (2011a). Clinical significance of vasculogenic mimicry in human gliomas. Journal of neuro-oncology *105*, 173-179.
Liu, Z., Li, Y., Zhao, W., Ma, Y., and Yang, X. (2011b). Demonstration of vasculogenic mimicry in astrocytomas and effects of Endostar on U251 cells. Pathology, research and practice *207*, 645-651.
Lozano-Romero, A., Astudillo-de la Vega, H., Terrones-Gurrola, M. C. D. R., Marchat, L. A., Hernández-Sotelo, D., Salinas-Vera, Y. M., Ramos-Payan, R., Silva-Cázares, M. B., Nuñez-Olvera, S. I., Hernández-de la Cruz, O. N., and López-Camarillo, C. (2020). HOX Transcript Antisense RNA. Noncoding RNA *6*.
Lu, J., Lin, Y., Li, F., Ye, H., Zhou, R., Jin, Y., Li, B., Xiong, X., and Cheng, N. (2018). MiR-205 suppresses tumor growth, invasion, and epithelial-mesenchymal transition by targeting SEMA4C in hepatocellular carcinoma. FASEB J, fj201800113R.
Ma, J. L., Han, S. X., Zhu, Q., Zhao, J., Zhang, D., Wang, L., and Lv, Y. (2011). Role of Twist in vasculogenic mimicry formation in hypoxic hepatocellular carcinoma cells in vitro. Biochem Biophys Res Commun *408*, 686-691.
Ma, Y., Zhang, H., Xiong, C., Liu, Z., Xu, Q., Feng, J., Zhang, J., Wang, Z., and Yan, X. (2018). CD146 mediates an E-cadherin-to-N-cadherin switch during TGF-β signaling-induced epithelial-mesenchymal transition. Cancer Lett *430*, 201-214.
Maiti, A., Qi, Q., Peng, X., Yan, L., Takabe, K., and Hait, N. C. (2019). Class I histone deacetylase inhibitor suppresses vasculogenic mimicry by enhancing the expression of tumor suppressor and anti-angiogenesis genes in aggressive human TNBC cells. Int J Oncol *55*, 116-130.
Maniotis, A. J., Folberg, R., Hess, A., Seftor, E. A., Gardner, L. M., Pe'er, J., Trent, J. M., Meltzer, P. S., and Hendrix, M. J. (1999). Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. Am J Pathol *155*, 739-752.
Mao, Y., Zhu, L., Huang, Z., Luo, C., Zhou, T., Li, L., Wang, G., Yang, Z., Qi, W., Yang, X., and Gao, G. (2020). Stem-like tumor cells involved in heterogeneous vasculogenesis in breast cancer. Endocr Relat Cancer *27*, 23-39.
Massi, D., Tarantini, F., Franchi, A., Paglierani, M., Di Serio, C., Pellerito, S., Leoncini, G., Cirino, G., Geppetti, P., and Santucci, M. (2006). Evidence for differential expression of Notch receptors and their ligands in melanocytic nevi and cutaneous malignant melanoma. Mod Pathol *19*, 246-254.
McDonald, D. M., Munn, L., and Jain, R. K. (2000). Vasculogenic mimicry: how convincing, how novel, and how significant? Am J Pathol *156*, 383-388.
Mei, X., Chen, Y. S., Chen, F. R., Xi, S. Y., and Chen, Z. P. (2017). Glioblastoma stem cell differentiation into endothelial cells evidenced through live-cell imaging. Neuro Oncol *19*, 1109-1118.
Meng, J., Sun, B., Zhao, X., Zhang, D., Gu, Q., Dong, X., Zhao, N., Liu, P., and Liu, Y. (2014). Doxycycline as an inhibitor of the epithelial-to-mesenchymal transition and vasculogenic mimicry in hepatocellular carcinoma. Mol Cancer Ther *13*, 3107-3122.
Miro, C., Di Cicco, E., Ambrosio, R., Mancino, G., Di Girolamo, D., Cicatiello, A. G., Sagliocchi, S., Nappi, A., De Stefano, M. A., Luongo, C.*, et al.* (2019). Thyroid hormone induces progression and invasiveness of squamous cell carcinomas by promoting a ZEB-1/E-cadherin switch. Nat Commun *10*, 5410.

Nakamura, R., Kataoka, H., Sato, N., Kanamori, M., Ihara, M., Igarashi, H., Ravshanov, S., Wang, Y. J., Li, Z. Y., Shimamura, T.*, et al.* (2005). EPHA2/EFNA1 expression in human gastric cancer. Cancer Sci *96*, 42-47.
Nieto, M. A., Huang, R. Y., Jackson, R. A., and Thiery, J. P. (2016). EMT: 2016. Cell *166*, 21-45.
Nievergall, E., Janes, P. W., Stegmayer, C., Vail, M. E., Haj, F. G., Teng, S. W., Neel, B. G., Bastiaens, P. I., and Lackmann, M. (2010). PTP1B regulates Eph receptor function and trafficking. J Cell Biol *191*, 1189-1203.
Orsenigo, F., Giampietro, C., Ferrari, A., Corada, M., Galaup, A., Sigismund, S., Ristagno, G., Maddaluno, L., Koh, G. Y., Franco, D.*, et al.* (2012). Phosphorylation of VE-cadherin is modulated by haemodynamic forces and contributes to the regulation of vascular permeability in vivo. Nat Commun *3*, 1208.
Pan, J. X., Wang, F., and Ye, L. Y. (2016). Doxorubicin-induced epithelial-mesenchymal transition through SEMA 4A in hepatocellular carcinoma. Biochem Biophys Res Commun *479*, 610-614.
Parri, M., Taddei, M. L., Bianchini, F., Calorini, L., and Chiarugi, P. (2009). EphA2 reexpression prompts invasion of melanoma cells shifting from mesenchymal to amoeboid-like motility style. Cancer Res *69*, 2072-2081.
Pastorino, O., Gentile, M. T., Mancini, A., Del Gaudio, N., Di Costanzo, A., Bajetto, A., Franco, P., Altucci, L., Florio, T., Stoppelli, M. P., and Colucci-D'Amato, L. (2019). Histone Deacetylase Inhibitors Impair Vasculogenic Mimicry from Glioblastoma Cells. Cancers (Basel) *11*.
Pastushenko, I., and Blanpain, C. (2019). EMT Transition States during Tumor Progression and Metastasis. Trends Cell Biol *29*, 212-226.
Pautu, V., Mellinger, A., Resnier, P., Lepeltier, E., Martin, L., Boussemart, L., Letournel, F., Passirani, C., and Clere, N. (2019). Melanoma tumour vasculature heterogeneity: from mice models to human. J Cancer Res Clin Oncol *145*, 589-597.
Pavon, L. F., Sibov, T. T., de Souza, A. V., da Cruz, E. F., Malheiros, S. M. F., Cabral, F. R., de Souza, J. G., Boufleur, P., de Oliveira, D. M., de Toledo, S. R. C.*, et al.* (2018). Tropism of mesenchymal stem cell toward CD133. Stem Cell Res Ther *9*, 310.
Peinado, H., Del Carmen Iglesias-de la Cruz, M., Olmeda, D., Csiszar, K., Fong, K. S., Vega, S., Nieto, M. A., Cano, A., and Portillo, F. (2005). A molecular role for lysyl oxidase-like 2 enzyme in snail regulation and tumor progression. EMBO J *24*, 3446-3458.
Peitzsch, C., Tyutyunnykova, A., Pantel, K., and Dubrovska, A. (2017). Cancer stem cells: The root of tumor recurrence and metastases. Semin Cancer Biol *44*, 10-24.
Peng, Z., Wang, J., Shan, B., Li, B., Peng, W., Dong, Y., Shi, W., Zhao, W., He, D., Duan, M.*, et al.* (2018). The long noncoding RNA LINC00312 induces lung adenocarcinoma migration and vasculogenic mimicry through directly binding YBX1. Mol Cancer *17*, 167.
Prieto-Vila, M., Yan, T., Calle, A. S., Nair, N., Hurley, L., Kasai, T., Kakuta, H., Masuda, J., Murakami, H., Mizutani, A., and Seno, M. (2016). iPSC-derived cancer stem cells provide a model of tumor vasculature. Am J Cancer Res *6*, 1906-1921.
Qi, L., Song, W., Liu, Z., Zhao, X., Cao, W., and Sun, B. (2015). Wnt3a Promotes the Vasculogenic Mimicry Formation of Colon Cancer via Wnt/β-Catenin Signaling. Int J Mol Sci *16*, 18564-18579.
Qian, B. Z., and Pollard, J. W. (2010). Macrophage diversity enhances tumor progression and metastasis. Cell *141*, 39-51.
Qin, Y., Zhao, W., Cheng, L., Wu, S., Wu, Q., Gao, J., Bian, Z., and Ma, L. (2019). Clinical significance of vasculogenic mimicry, vascular endothelial cadherin and SOX4 in patients with esophageal squamous cell carcinoma. Int J Clin Exp Pathol *12*, 2462-2473.

Rai, A., and Cross, J. C. (2014). Development of the hemochorial maternal vascular spaces in the placenta through endothelial and vasculogenic mimicry. Dev Biol *387*, 131-141.
Ren, D., Zhu, X., Kong, R., Zhao, Z., Sheng, J., Wang, J., Xu, X., Liu, J., Cui, K., Zhang, X. H.*, et al.* (2018). Targeting Brain-Adaptive Cancer Stem Cells Prohibits Brain Metastatic Colonization of Triple-Negative Breast Cancer. Cancer Res *78*, 2052-2064.
Ribatti, D., Tamma, R., and Annese, T. (2020). Epithelial-Mesenchymal Transition in Cancer: A Historical Overview. Transl Oncol *13*, 100773.
Ricci-Vitiani, L., Pallini, R., Biffoni, M., Todaro, M., Invernici, G., Cenci, T., Maira, G., Parati, E. A., Stassi, G., Larocca, L. M., and De Maria, R. (2010). Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. Nature *468*, 824-828.
Robertson, F. M., Simeone, A. M., Lucci, A., McMurray, J. S., Ghosh, S., and Cristofanilli, M. (2010). Differential regulation of the aggressive phenotype of inflammatory breast cancer cells by prostanoid receptors EP3 and EP4. Cancer *116*, 2806-2814.
Rong, X., Huang, B., Qiu, S., Li, X., He, L., and Peng, Y. (2016). Tumor-associated macrophages induce vasculogenic mimicry of glioblastoma multiforme through cyclooxygenase-2 activation. Oncotarget *7*, 83976-83986.
Ruf, W., Seftor, E. A., Petrovan, R. J., Weiss, R. M., Gruman, L. M., Margaryan, N. V., Seftor, R. E., Miyagi, Y., and Hendrix, M. J. (2003). Differential role of tissue factor pathway inhibitors 1 and 2 in melanoma vasculogenic mimicry. Cancer Res *63*, 5381-5389.
Sabet, O., Stockert, R., Xouri, G., Brüggemann, Y., Stanoev, A., and Bastiaens, P. I. H. (2015). Ubiquitination switches EphA2 vesicular traffic from a continuous safeguard to a finite signalling mode. Nat Commun *6*, 8047.
Salinas-Vera, Y. M., Gallardo-Rincón, D., García-Vázquez, R., Hernández-de la Cruz, O. N., Marchat, L. A., González-Barrios, J. A., Ruíz-García, E., Vázquez-Calzada, C., Contreras-Sanzón, E., Resendiz-Hernández, M.*, et al.* (2019). HypoxamiRs Profiling Identify miR-745 as a Regulator of the Early Stages of Vasculogenic Mimicry in SKOV3 Ovarian Cancer Cells. Front Oncol *9*, 381.
Salinas-Vera, Y. M., Marchat, L. A., García-Vázquez, R., González de la Rosa, C. H., Castañeda-Saucedo, E., Tito, N. N., Flores, C. P., Pérez-Plasencia, C., Cruz-Colin, J. L., Carlos-Reyes, Á.*, et al.* (2018). Cooperative multi-targeting of signaling networks by angiomiR-204 inhibits vasculogenic mimicry in breast cancer cells. Cancer Lett *432*, 17-27.
Sannino, G., Marchetto, A., Kirchner, T., and Grünewald, T. G. P. (2017). Epithelial-to-Mesenchymal and Mesenchymal-to-Epithelial Transition in Mesenchymal Tumors: A Paradox in Sarcomas? Cancer Res *77*, 4556-4561.
Saxena, S., Purohit, A., Varney, M. L., Hayashi, Y., and Singh, R. K. (2018). Semaphorin-5A maintains epithelial phenotype of malignant pancreatic cancer cells. BMC Cancer *18*, 1283.
Schatton, T., Murphy, G. F., Frank, N. Y., Yamaura, K., Waaga-Gasser, A. M., Gasser, M., Zhan, Q., Jordan, S., Duncan, L. M., Weishaupt, C.*, et al.* (2008). Identification of cells initiating human melanomas. Nature *451*, 345-349.
Schnegg, C. I., Yang, M. H., Ghosh, S. K., and Hsu, M. Y. (2015). Induction of Vasculogenic Mimicry Overrides VEGF-A Silencing and Enriches Stem-like Cancer Cells in Melanoma. Cancer Res *75*, 1682-1690.
Scully, S., Francescone, R., Faibish, M., Bentley, B., Taylor, S. L., Oh, D., Schapiro, R., Moral, L., Yan, W., and Shao, R. (2012). Transdifferentiation of glioblastoma stem-like cells into mural cells drives vasculogenic mimicry in glioblastomas. J Neurosci *32*, 12950-12960.
Seftor, E. A., Meltzer, P. S., Schatteman, G. C., Gruman, L. M., Hess, A. R., Kirschmann, D. A., Seftor, R. E., and Hendrix, M. J. (2002). Expression of multiple molecular phenotypes by

aggressive melanoma tumor cells: role in vasculogenic mimicry. Crit Rev Oncol Hematol *44*, 17-27.
Seftor, R. E., Seftor, E. A., Koshikawa, N., Meltzer, P. S., Gardner, L. M., Bilban, M., Stetler-Stevenson, W. G., Quaranta, V., and Hendrix, M. J. (2001). Cooperative interactions of laminin 5 gamma2 chain, matrix metalloproteinase-2, and membrane type-1-matrix/metalloproteinase are required for mimicry of embryonic vasculogenesis by aggressive melanoma. Cancer Res *61*, 6322-6327.
Shao, B., Zhao, X., Liu, T., Zhang, Y., Sun, R., Dong, X., Liu, F., Zhao, N., Zhang, D., Wu, L.*, et al.* (2019). LOXL2 promotes vasculogenic mimicry and tumour aggressiveness in hepatocellular carcinoma. J Cell Mol Med *23*, 1363-1374.
Shetti, D., Zhang, B., Fan, C., Mo, C., Lee, B. H., and Wei, K. (2019). Low Dose of Paclitaxel Combined with XAV939 Attenuates Metastasis, Angiogenesis and Growth in Breast Cancer by Suppressing Wnt Signaling. Cells *8*.
Shih, I. M. (2011). Trophoblastic vasculogenic mimicry in gestational choriocarcinoma. Mod Pathol *24*, 646-652.
Shuai, Q., Cao, L., Qin, Z., Zhang, Y., Gu, Z., and Yang, J. (2020). VE-cadherin fusion protein substrate enhanced the vasculogenic mimicry capability of hepatocellular carcinoma cells. J Mater Chem B *8*, 1699-1712.
Song, J., and Li, Y. (2017). miR-25-3p reverses epithelial-mesenchymal transition via targeting Sema4C in cisplatin-resistance cervical cancer cells. Cancer Sci *108*, 23-31.
Song, Y., Mu, L., Han, X., Li, Q., Dong, B., Li, H., and Liu, X. (2013). MicroRNA-9 inhibits vasculogenic mimicry of glioma cell lines by suppressing Stathmin expression. J Neurooncol *115*, 381-390.
Sood, A. K., Seftor, E. A., Fletcher, M. S., Gardner, L. M., Heidger, P. M., Buller, R. E., Seftor, R. E., and Hendrix, M. J. (2001). Molecular determinants of ovarian cancer plasticity. Am J Pathol *158*, 1279-1288.
Spinella, F., Caprara, V., Di Castro, V., Rosanò, L., Cianfrocca, R., Natali, P. G., and Bagnato, A. (2013). Endothelin-1 induces the transactivation of vascular endothelial growth factor receptor-3 and modulates cell migration and vasculogenic mimicry in melanoma cells. J Mol Med (Berl) *91*, 395-405.
Su, S., Chen, J., Yao, H., Liu, J., Yu, S., Lao, L., Wang, M., Luo, M., Xing, Y., Chen, F.*, et al.* (2018). CD10. Cell *172*, 841-856.e816.
Sun, D., Sun, B., Liu, T., Zhao, X., Che, N., Gu, Q., Dong, X., Yao, Z., Li, R., Li, J.*, et al.* (2013). Slug promoted vasculogenic mimicry in hepatocellular carcinoma. J Cell Mol Med *17*, 1038-1047.
Sun, H., Yao, N., Cheng, S., Li, L., Liu, S., Yang, Z., Shang, G., Zhang, D., and Yao, Z. (2019). Cancer stem-like cells directly participate in vasculogenic mimicry channels in triple-negative breast cancer. Cancer Biol Med *16*, 299-311.
Sun, H., Zhang, D., Yao, Z., Lin, X., Liu, J., Gu, Q., Dong, X., Liu, F., Wang, Y., Yao, N.*, et al.* (2017). Anti-angiogenic treatment promotes triple-negative breast cancer invasion via vasculogenic mimicry. Cancer Biol Ther *18*, 205-213.
Sun, Q., Zou, X., Zhang, T., Shen, J., Yin, Y., and Xiang, J. (2014). The role of miR-200a in vasculogenic mimicry and its clinical significance in ovarian cancer. Gynecol Oncol *132*, 730-738.
Sun, T., Zhao, N., Zhao, X. L., Gu, Q., Zhang, S. W., Che, N., Wang, X. H., Du, J., Liu, Y. X., and Sun, B. C. (2010). Expression and functional significance of Twist1 in hepatocellular carcinoma: its role in vasculogenic mimicry. Hepatology *51*, 545-556.

Sun, Y., Yue, S., Wang, Y., and Lu, F. (2018). Histone Deacetylase Inhibitors in Cancer Therapy. Curr Top Med Chem *18*, 2420-2428.
Takasugi, M., Okada, R., Takahashi, A., Virya Chen, D., Watanabe, S., and Hara, E. (2017). Small extracellular vesicles secreted from senescent cells promote cancer cell proliferation through EphA2. Nat Commun *8*, 15729.
Tam, K. J., Hui, D. H. F., Lee, W. W., Dong, M., Tombe, T., Jiao, I. Z. F., Khosravi, S., Takeuchi, A., Peacock, J. W., Ivanova, L*., et al.* (2017). Semaphorin 3 C drives epithelial-to-mesenchymal transition, invasiveness, and stem-like characteristics in prostate cells. Sci Rep *7*, 11501.
Tao, W., Sun, W., Zhu, H., and Zhang, J. (2018). Knockdown of long non-coding RNA TP73-AS1 suppresses triple negative breast cancer cell vasculogenic mimicry by targeting miR-490-3p/TWIST1 axis. Biochem Biophys Res Commun *504*, 629-634.
Thiery, J. P. (2002). Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer *2*, 442-454.
Thijssen, V. L., Paulis, Y. W., Nowak-Sliwinska, P., Deumelandt, K. L., Hosaka, K., Soetekouw, P. M., Cimpean, A. M., Raica, M., Pauwels, P., van den Oord, J. J*., et al.* (2018). Targeting PDGF-mediated recruitment of pericytes blocks vascular mimicry and tumor growth. J Pathol *246*, 447-458.
Tkach, M., and Thery, C. (2016). Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. Cell *164*, 1226-1232.
Tomita, H., Tanaka, K., Tanaka, T., and Hara, A. (2016). Aldehyde dehydrogenase 1A1 in stem cells and cancer. Oncotarget *7*, 11018-11032.
Topczewska, J. M., Postovit, L. M., Margaryan, N. V., Sam, A., Hess, A. R., Wheaton, W. W., Nickoloff, B. J., Topczewski, J., and Hendrix, M. J. (2006). Embryonic and tumorigenic pathways converge via Nodal signaling: role in melanoma aggressiveness. Nat Med *12*, 925-932.
Treps, L., Le Guelte, A., and Gavard, J. (2013). Emerging roles of Semaphorins in the regulation of epithelial and endothelial junctions. Tissue Barriers *1*, e23272.
Tseng, C. H., Murray, K. D., Jou, M. F., Hsu, S. M., Cheng, H. J., and Huang, P. H. (2011). Sema3E/plexin-D1 mediated epithelial-to-mesenchymal transition in ovarian endometrioid cancer. PLoS One *6*, e19396.
Valdivia, A., Mingo, G., Aldana, V., Pinto, M. P., Ramirez, M., Retamal, C., Gonzalez, A., Nualart, F., Corvalan, A. H., and Owen, G. I. (2019). Fact or Fiction, It Is Time for a Verdict on Vasculogenic Mimicry? Front Oncol *9*, 680.
van der Schaft, D. W., Hillen, F., Pauwels, P., Kirschmann, D. A., Castermans, K., Egbrink, M. G., Tran, M. G., Sciot, R., Hauben, E., Hogendoorn, P. C*., et al.* (2005). Tumor cell plasticity in Ewing sarcoma, an alternative circulatory system stimulated by hypoxia. Cancer Res *65*, 11520-11528.
Vartanian, A., Gatsina, G., Grigorieva, I., Solomko, E., Dombrovsky, V., Baryshnikov, A., and Stepanova, E. (2013). The involvement of Notch signaling in melanoma vasculogenic mimicry. Clin Exp Med *13*, 201-209.
Vartanian, A., Stepanova, E., Grigorieva, I., Solomko, E., Baryshnikov, A., and Lichinitser, M. (2011). VEGFR1 and PKCα signaling control melanoma vasculogenic mimicry in a VEGFR2 kinase-independent manner. Melanoma Res *21*, 91-98.
Virant-Klun, I., Skerl, P., Novakovic, S., Vrtacnik-Bokal, E., and Smrkolj, S. (2019). Similar Population of CD133+ and DDX4+ VSEL-Like Stem Cells Sorted from Human Embryonic Stem Cell, Ovarian, and Ovarian Cancer Ascites Cell Cultures: The Real Embryonic Stem Cells? Cells *8*.

Vishnubalaji, R., Manikandan, M., Fahad, M., Hamam, R., Alfayez, M., Kassem, M., Aldahmash, A., and Alajez, N. M. (2018). Molecular profiling of ALDH1. Oncotarget *9*, 13551-13564.
Wagenblast, E., Soto, M., Gutiérrez-Ángel, S., Hartl, C. A., Gable, A. L., Maceli, A. R., Erard, N., Williams, A. M., Kim, S. Y., Dickopf, S.*, et al.* (2015). A model of breast cancer heterogeneity reveals vascular mimicry as a driver of metastasis. Nature *520*, 358-362.
Walker-Daniels, J., Riese, D. J., and Kinch, M. S. (2002). c-Cbl-dependent EphA2 protein degradation is induced by ligand binding. Mol Cancer Res *1*, 79-87.
Wang, H., Sun, W., Zhang, W. Z., Ge, C. Y., Zhang, J. T., Liu, Z. Y., and Fan, Y. Z. (2014). Inhibition of tumor vasculogenic mimicry and prolongation of host survival in highly aggressive gallbladder cancers by norcantharidin via blocking the ephrin type a receptor 2/focal adhesion kinase/paxillin signaling pathway. PLoS One *9*, e96982.
Wang, H. F., Wang, S. S., Zheng, M., Dai, L. L., Wang, K., Gao, X. L., Cao, M. X., Yu, X. H., Pang, X., Zhang, M.*, et al.* (2019). Hypoxia promotes vasculogenic mimicry formation by vascular endothelial growth factor A mediating epithelial-mesenchymal transition in salivary adenoid cystic carcinoma. Cell Prolif *52*, e12600.
Wang, L., Song, Y., Wang, H., Liu, K., Shao, Z., and Shang, Z. (2020a). MiR-210-3p-EphrinA3-PI3K/AKT axis regulates the progression of oral cancer. J Cell Mol Med *24*, 4011-4022.
Wang, M., Yang, C., Liu, X., Zheng, J., Xue, Y., Ruan, X., Shen, S., Wang, D., Li, Z., Cai, H., and Liu, Y. (2020b). An upstream open reading frame regulates vasculogenic mimicry of glioma via ZNRD1-AS1/miR-499a-5p/ELF1/EMI1 pathway. J Cell Mol Med.
Wang, M., Zhao, X., Zhu, D., Liu, T., Liang, X., Liu, F., Zhang, Y., Dong, X., and Sun, B. (2017). HIF-1α promoted vasculogenic mimicry formation in hepatocellular carcinoma through LOXL2 up-regulation in hypoxic tumor microenvironment. J Exp Clin Cancer Res *36*, 60.
Wang, R., Chadalavada, K., Wilshire, J., Kowalik, U., Hovinga, K. E., Geber, A., Fligelman, B., Leversha, M., Brennan, C., and Tabar, V. (2010). Glioblastoma stem-like cells give rise to tumour endothelium. Nature *468*, 829-833.
Wang, S. Y., Ke, Y. Q., Lu, G. H., Song, Z. H., Yu, L., Xiao, S., Sun, X. L., Jiang, X. D., Yang, Z. L., and Hu, C. C. (2013). Vasculogenic mimicry is a prognostic factor for postoperative survival in patients with glioblastoma. J Neurooncol *112*, 339-345.
Wang, S. Y., Yu, L., Ling, G. Q., Xiao, S., Sun, X. L., Song, Z. H., Liu, Y. J., Jiang, X. D., Cai, Y. Q., and Ke, Y. Q. (2012). Vasculogenic mimicry and its clinical significance in medulloblastoma. Cancer Biol Ther *13*, 341-348.
Wang, Y., Yang, R., Wang, X., Ci, H., Zhou, L., Zhu, B., Wu, S., and Wang, D. (2018). Evaluation of the correlation of vasculogenic mimicry, Notch4, DLL4, and KAI1/CD82 in the prediction of metastasis and prognosis in non-small cell lung cancer. Medicine (Baltimore) *97*, e13817.
Wang, Z., Chen, J., Zhang, W., Zheng, Y., Liu, L., Wu, H., Ye, J., Qi, B., Wu, Y., and Song, X. (2016). Axon guidance molecule semaphorin3A is a novel tumor suppressor in head and neck squamous cell carcinoma. Oncotarget *7*, 6048-6062.
Wendt, M. K., Allington, T. M., and Schiemann, W. P. (2009). Mechanisms of the epithelial-mesenchymal transition by TGF-beta. Future oncology *5*, 1145-1168.
Wheelock, M. J., Shintani, Y., Maeda, M., Fukumoto, Y., and Johnson, K. R. (2008). Cadherin switching. J Cell Sci *121*, 727-735.
Wigerup, C., Påhlman, S., and Bexell, D. (2016). Therapeutic targeting of hypoxia and hypoxia-inducible factors in cancer. Pharmacol Ther *164*, 152-169.
Williamson, S. C., Metcalf, R. L., Trapani, F., Mohan, S., Antonello, J., Abbott, B., Leong, H. S., Chester, C. P., Simms, N., Polanski, R.*, et al.* (2016). Vasculogenic mimicry in small cell lung cancer. Nat Commun *7*, 13322.

Wu, H. B., Yang, S., Weng, H. Y., Chen, Q., Zhao, X. L., Fu, W. J., Niu, Q., Ping, Y. F., Wang, J. M., Zhang, X.*, et al.* (2017). Autophagy-induced KDR/VEGFR-2 activation promotes the formation of vasculogenic mimicry by glioma stem cells. Autophagy *13*, 1528-1542.

Wu, N., Zhao, X., Liu, M., Liu, H., Yao, W., Zhang, Y., Cao, S., and Lin, X. (2011). Role of microRNA-26b in glioma development and its mediated regulation on EphA2. PLoS One *6*, e16264.

Wurmser, A. E., Nakashima, K., Summers, R. G., Toni, N., D'Amour, K. A., Lie, D. C., and Gage, F. H. (2004). Cell fusion-independent differentiation of neural stem cells to the endothelial lineage. Nature *430*, 350-356.

Xia, Y., Cai, X., Fan, J., Zhang, L., Li, Z., Ren, J., Wu, G., and Zhu, F. (2017). RhoA/ROCK pathway inhibition by fasudil suppresses the vasculogenic mimicry of U2OS osteosarcoma cells in vitro. Anticancer Drugs *28*, 514-521.

Xia, Y., Cai, X. Y., Fan, J. Q., Zhang, L. L., Ren, J. H., Chen, J., Li, Z. Y., Zhang, R. G., Zhu, F., and Wu, G. (2015). Rho Kinase Inhibitor Fasudil Suppresses the Vasculogenic Mimicry of B16 Mouse Melanoma Cells Both In Vitro and In Vivo. Mol Cancer Ther *14*, 1582-1590.

Xia, Y., Cai, X. Y., Fan, J. Q., Zhang, L. L., Ren, J. H., Li, Z. Y., Zhang, R. G., Zhu, F., and Wu, G. (2019). The role of sema4D in vasculogenic mimicry formation in non-small cell lung cancer and the underlying mechanisms. Int J Cancer *144*, 2227-2238.

Xiang, T., Lin, Y. X., Ma, W., Zhang, H. J., Chen, K. M., He, G. P., Zhang, X., Xu, M., Feng, Q. S., Chen, M. Y.*, et al.* (2018). Vasculogenic mimicry formation in EBV-associated epithelial malignancies. Nat Commun *9*, 5009.

Xiao, T., Zhong, W., Zhao, J., Qian, B., Liu, H., Chen, S., Qiao, K., Lei, Y., Zong, S., Wang, H.*, et al.* (2018). Polyphyllin I suppresses the formation of vasculogenic mimicry via Twist1/VE-cadherin pathway. Cell Death Dis *9*, 906.

Xu, J., Zhang, Y., Wang, Y., Tao, X., Cheng, L., Wu, S., and Tao, Y. (2018a). Correlation of KAI1, CD133 and vasculogenic mimicry with the prediction of metastasis and prognosis in hepatocellular carcinoma. Int J Clin Exp Pathol *11*, 3638-3646.

Xu, M. R., Wei, P. F., Suo, M. Z., Hu, Y., Ding, W., Su, L., Zhu, Y. D., Song, W. J., Tang, G. H., Zhang, M., and Li, P. (2019). Brucine Suppresses Vasculogenic Mimicry in Human Triple-Negative Breast Cancer Cell Line MDA-MB-231. Biomed Res Int *2019*, 6543230.

Xu, S., Bai, J., Zhuan, Z., Li, B., Zhang, Z., Wu, X., Luo, X., and Yang, L. (2018b). EBV-LMP1 is involved in vasculogenic mimicry formation via VEGFA/VEGFR1 signaling in nasopharyngeal carcinoma. Oncol Rep *40*, 377-384.

Xu, S., Zhang, J., Xue, H., Guo, X., Han, X., Li, T., Gao, X., Liu, Q., and Li, G. (2017). MicroRNA-584-3p reduces the vasculogenic mimicry of human glioma cells by regulating hypoxia-induced ROCK1 dependent stress fiber formation. Neoplasma *64*, 13-21.

Xue, H., Gao, X., Xu, S., Zhang, J., Guo, X., Yan, S., Li, T., Liu, Q., and Li, G. (2016). MicroRNA-Let-7f reduces the vasculogenic mimicry of human glioma cells by regulating periostin-dependent migration. Oncol Rep *35*, 1771-1777.

Xue, Y., Sun, R., Zheng, W., Yang, L., and An, R. (2020). Forskolin promotes vasculogenic mimicry and invasion via Notch-1-activated epithelial-to-mesenchymal transition in syncytiolization of trophoblast cells in choriocarcinoma. Int J Oncol *56*, 1129-1139.

Yang, F., Wen, M., Pan, D., Lin, X., Mo, J., Dong, X., Liao, S., and Ma, Y. (2019). IL-33/ST2 Axis Regulates Vasculogenic Mimicry via ERK1/2-MMP-2/9 Pathway in Melanoma. Dermatology *235*, 225-233.

Yang, J., Lu, Y., Lin, Y. Y., Zheng, Z. Y., Fang, J. H., He, S., and Zhuang, S. M. (2016). Vascular mimicry formation is promoted by paracrine TGF-β and SDF1 of cancer-associated fibroblasts and inhibited by miR-101 in hepatocellular carcinoma. Cancer Lett *383*, 18-27.

Yang, Q., Wang, Y., Lu, X., Zhao, Z., Zhu, L., Chen, S., Wu, Q., Chen, C., and Wang, Z. (2015a). MiR-125b regulates epithelial-mesenchymal transition via targeting Sema4C in paclitaxel-resistant breast cancer cells. Oncotarget *6*, 3268-3279.

Yang, S., Liu, Y., Li, M. Y., Ng, C. S. H., Yang, S. L., Wang, S., Zou, C., Dong, Y., Du, J., Long, X*., et al.* (2017). FOXP3 promotes tumor growth and metastasis by activating Wnt/β-catenin signaling pathway and EMT in non-small cell lung cancer. Mol Cancer *16*, 124.

Yang, Y. H., Markus, M. A., Mangs, A. H., Raitskin, O., Sperling, R., and Morris, B. J. (2013). ZRANB2 localizes to supraspliceosomes and influences the alternative splicing of multiple genes in the transcriptome. Mol Biol Rep *40*, 5381-5395.

Yang, Z., Sun, B., Li, Y., Zhao, X., Gu, Q., An, J., Dong, X., Liu, F., and Wang, Y. (2015b). ZEB2 promotes vasculogenic mimicry by TGF-β1 induced epithelial-to-mesenchymal transition in hepatocellular carcinoma. Exp Mol Pathol *98*, 352-359.

Yao, N., Ren, K., Gu, X. J., Wu, S. J., Shi, X., Chang, Q., Li, Y. G., Gao, Z. X., Jin, Q. M., Zhang, J*., et al.* (2020). Identification of potential crucial genes associated with vasculogenic mimicry in human osteosarcoma based on gene expression profile. Neoplasma *67*, 286-295.

Yao, X., Ping, Y., Liu, Y., Chen, K., Yoshimura, T., Liu, M., Gong, W., Chen, C., Niu, Q., Guo, D*., et al.* (2013). Vascular endothelial growth factor receptor 2 (VEGFR-2) plays a key role in vasculogenic mimicry formation, neovascularization and tumor initiation by Glioma stem-like cells. PLoS One *8*, e57188.

Yin, A. H., Miraglia, S., Zanjani, E. D., Almeida-Porada, G., Ogawa, M., Leary, A. G., Olweus, J., Kearney, J., and Buck, D. W. (1997). AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood *90*, 5002-5012.

Yu, W., Ding, J., He, M., Chen, Y., Wang, R., Han, Z., Xing, E. Z., Zhang, C., and Yeh, S. (2019). Estrogen receptor β promotes the vasculogenic mimicry (VM) and cell invasion via altering the lncRNA-MALAT1/miR-145-5p/NEDD9 signals in lung cancer. Oncogene *38*, 1225-1238.

Yue, W. Y., and Chen, Z. P. (2005). Does vasculogenic mimicry exist in astrocytoma? J Histochem Cytochem *53*, 997-1002.

Yue, Z., Yuan, Z., Zeng, L., Wang, Y., Lai, L., Li, J., Sun, P., Xue, X., Qi, J., Yang, Z*., et al.* (2018). LGR4 modulates breast cancer initiation, metastasis, and cancer stem cells. FASEB J *32*, 2422-2437.

Zavros, Y. (2017). Initiation and Maintenance of Gastric Cancer: A Focus on CD44 Variant Isoforms and Cancer Stem Cells. Cell Mol Gastroenterol Hepatol *4*, 55-63.

Zhang, C., Chen, W., Zhang, X., Huang, B., Chen, A., He, Y., Wang, J., and Li, X. (2016). Galunisertib inhibits glioma vasculogenic mimicry formation induced by astrocytes. Sci Rep *6*, 23056.

Zhang, D., Sun, B., Zhao, X., Ma, Y., Ji, R., Gu, Q., Dong, X., Li, J., Liu, F., Jia, X*., et al.* (2014a). Twist1 expression induced by sunitinib accelerates tumor cell vasculogenic mimicry by increasing the population of CD133+ cells in triple-negative breast cancer. Mol Cancer *13*, 207.

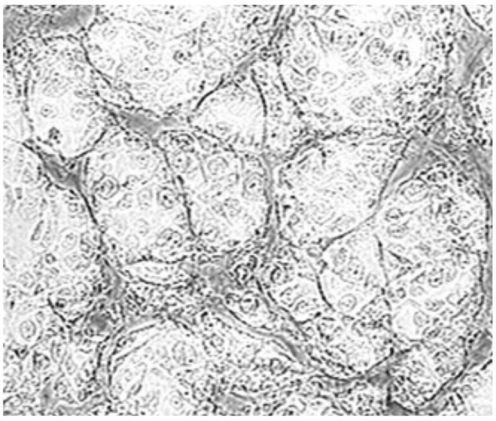
Zhang, D., Sun, B., Zhao, X., Sun, H., An, J., Lin, X., Zhu, D., Wang, X., Liu, F., Zhang, Y*., et al.* (2020). Twist1 accelerates tumour vasculogenic mimicry by inhibiting Claudin15 expression in triple-negative breast cancer. J Cell Mol Med.

Zhang, J. G., Li, X. Y., Wang, Y. Z., Zhang, Q. D., Gu, S. Y., Wu, X., Zhu, G. H., Li, Q., and Liu, G. L. (2014b). ROCK is involved in vasculogenic mimicry formation in hepatocellular carcinoma cell line. PLoS One *9*, e107661.

Zhang, J. G., Zhang, D. D., Wu, X., Wang, Y. Z., Gu, S. Y., Zhu, G. H., Li, X. Y., Li, Q., and Liu, G. L. (2015a). Incarvine C suppresses proliferation and vasculogenic mimicry of hepatocellular carcinoma cells via targeting ROCK inhibition. BMC Cancer *15*, 814.
Zhang, L., Xu, Y., Sun, J., Chen, W., Zhao, L., Ma, C., Wang, Q., Huang, B., Zhang, Y., Li, X., and Qu, X. (2017). M2-like tumor-associated macrophages drive vasculogenic mimicry through amplification of IL-6 expression in glioma cells. Oncotarget *8*, 819-832.
Zhang, S., Fu, Z., Wei, J., Guo, J., Liu, M., and Du, K. (2015b). Peroxiredoxin 2 is involved in vasculogenic mimicry formation by targeting VEGFR2 activation in colorectal cancer. Med Oncol *32*, 414.
Zhang, S., Li, M., Zhang, D., Xu, S., Wang, X., Liu, Z., Zhao, X., and Sun, B. (2009). Hypoxia influences linearly patterned programmed cell necrosis and tumor blood supply patterns formation in melanoma. Lab Invest *89*, 575-586.
Zhang, W., Zhou, P., Meng, A., Zhang, R., and Zhou, Y. (2018). Down-regulating Myoferlin inhibits the vasculogenic mimicry of melanoma via decreasing MMP-2 and inducing mesenchymal-to-epithelial transition. J Cell Mol Med *22*, 1743-1754.
Zhao, N., Sun, H., Sun, B., Zhu, D., Zhao, X., Wang, Y., Gu, Q., Dong, X., Liu, F., Zhang, Y., and Li, X. (2016a). miR-27a-3p suppresses tumor metastasis and VM by down-regulating VE-cadherin expression and inhibiting EMT: an essential role for Twist-1 in HCC. Sci Rep *6*, 23091.
Zhao, X., Sun, B., Li, Y., Liu, Y., Zhang, D., Wang, X., Gu, Q., Zhao, J., Dong, X., Liu, Z., and Che, N. (2015). Dual effects of collagenase-3 on melanoma: metastasis promotion and disruption of vasculogenic mimicry. Oncotarget *6*, 8890-8899.
Zhao, X., Sun, B., Liu, T., Shao, B., Sun, R., Zhu, D., Zhang, Y., Gu, Q., Dong, X., Liu, F*., et al.* (2018). Long noncoding RNA n339260 promotes vasculogenic mimicry and cancer stem cell development in hepatocellular carcinoma. Cancer Sci *109*, 3197-3208.
Zhao, X., Wang, Y., Deng, R., Zhang, H., Dou, J., Yuan, H., Hou, G., Du, Y., Chen, Q., and Yu, J. (2016b). miR186 suppresses prostate cancer progression by targeting Twist1. Oncotarget *7*, 33136-33151.
Zhao, Z., Xiang, S., Qi, J., Wei, Y., Zhang, M., Yao, J., Zhang, T., Meng, M., Wang, X., and Zhou, Q. (2020). Correction of the tumor suppressor Salvador homolog-1 deficiency in tumors by lycorine as a new strategy in lung cancer therapy. Cell Death Dis *11*, 387.
Zhong, Z., Yu, J., Virshup, D. M., and Madan, B. (2020). Wnts and the hallmarks of cancer. Cancer Metastasis Rev.
Zhou, J., Wang, H., Che, J., Xu, L., Yang, W., Li, Y., and Zhou, W. (2020). Silencing of microRNA-135b inhibits invasion, migration, and stemness of CD24. Cancer Cell Int *20*, 134.
Zhuo, M., Yuan, C., Han, T., Hu, H., Cui, J., Jiao, F., and Wang, L. (2019). JQ1 effectively inhibits vasculogenic mimicry of pancreatic ductal adenocarcinoma cells via the ERK1/2-MMP-2/9 signaling pathway both in vitro and in vivo. Am J Transl Res *11*, 1030-1039.
Zigrino, P., Kuhn, I., Bäuerle, T., Zamek, J., Fox, J. W., Neumann, S., Licht, A., Schorpp-Kistner, M., Angel, P., and Mauch, C. (2009). Stromal expression of MMP-13 is required for melanoma invasion and metastasis. J Invest Dermatol *129*, 2686-2693.

Figure 1

A Patterned vascular mimicry



B Tubular vascular mimicry

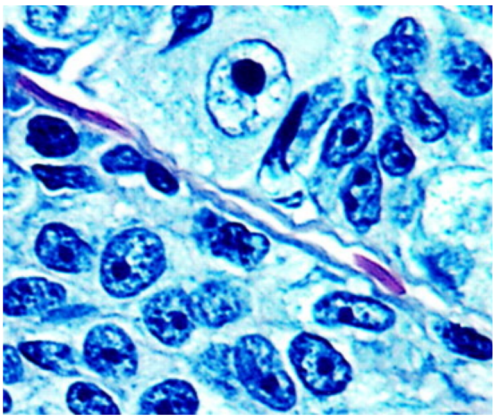


Figure 2

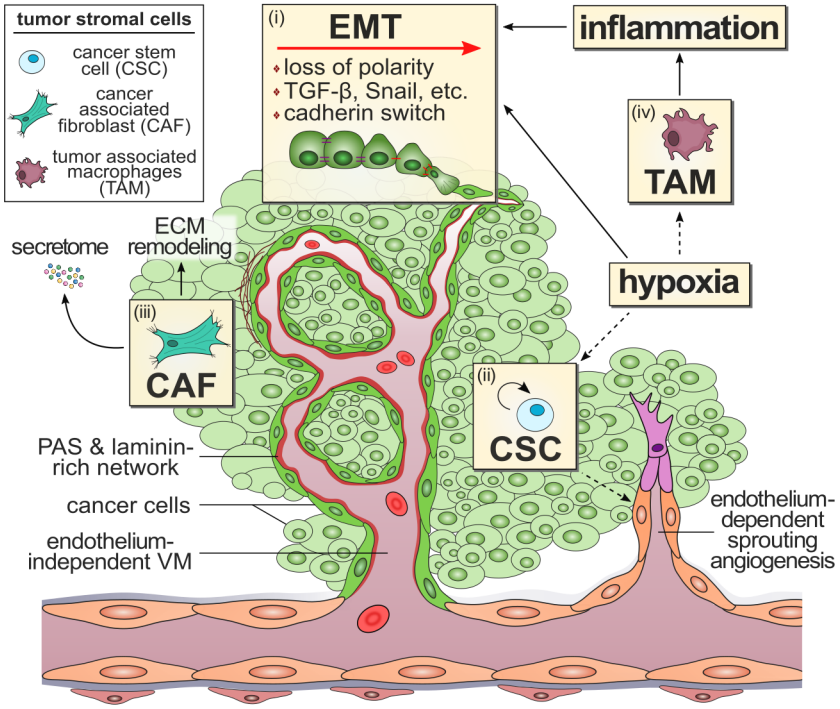
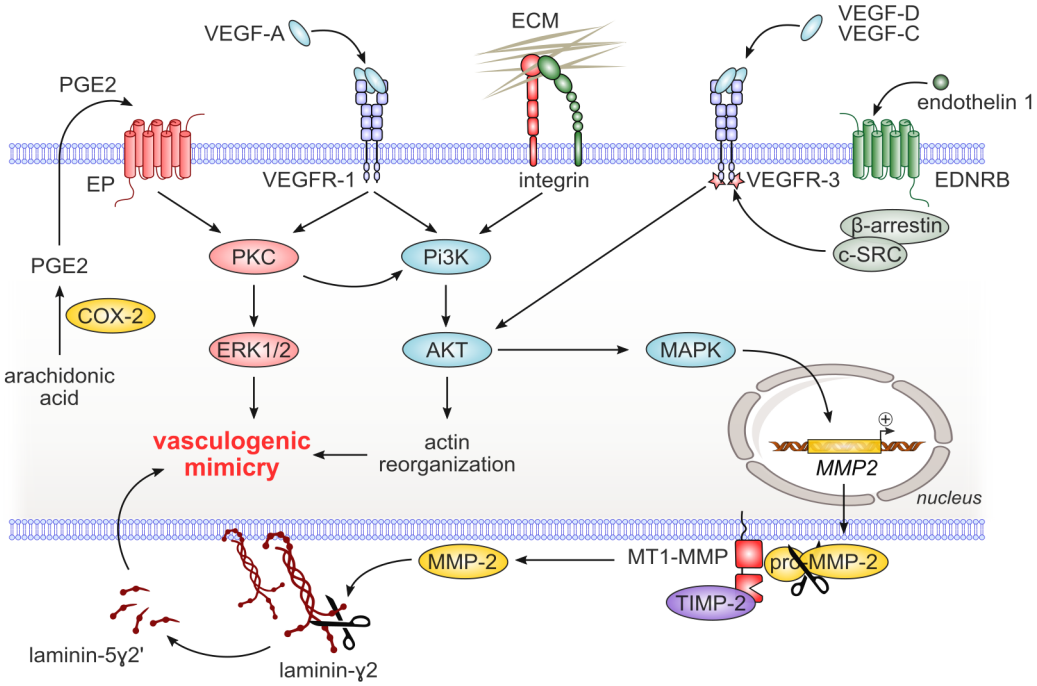

tumor stromal cells
cancer stem cell (CSC)
cancer associated fibroblast (CAF)
tumor associated macrophages (TAM)
(i)
EMT
loss of polarity
TGF-β, Snail, etc.
cadherin switch
inflammation
(iv)
TAM
hypoxia
secretome
ECM remodeling
(iii)
CAF
(ii)
CSC
PAS & laminin-rich network
cancer cells
endothelium-independent VM
endothelium-dependent sprouting angiogenesis

Figure 3

A VEGF/VEGFR-related signaling



B VE-cadherin-related signaling

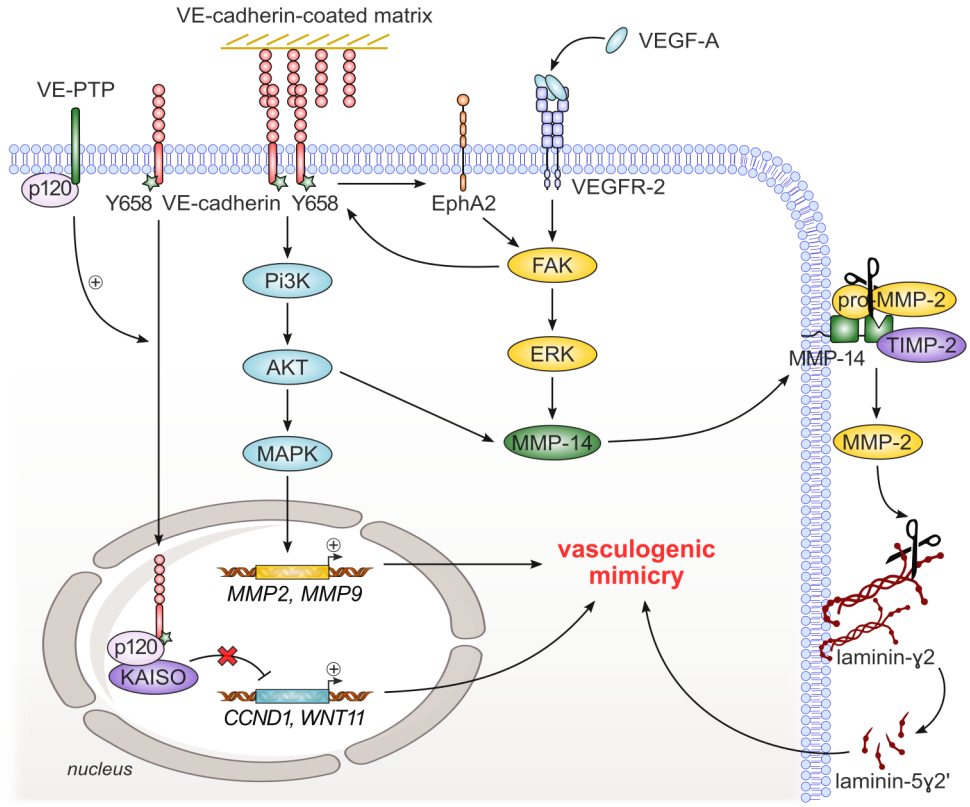


Table 1
Non-coding RNAs involved during vasculogenic mimicry.

ncRNAs	Cancer type	Signaling involved	miRNA involved	Effect on VM	References
miRNAs:					
miR-204	TNBC	PI3K/AKT, RAF1/MAPK, VEGF, and FAK/SRC signaling	n/a	suppress	(Salinas-Vera et al., 2018); (Lozano-Romero et al., 2020)
miR-490-3p	breast cancer; clear cell renal cell carcinoma	Twist1 and vimentin targeting	n/a	suppress	(Zhang, Li, et al., 2014b); (Tao et al., 2018)
miR-193b	breast cancer	DDAH1 targeting	n/a	suppress	(Hulin et al., 2017)
miR-124	breast cancer; cervical cancer	Slug targeting; interact with AmotL1 to reduce invasion and VM	n/a	suppress	(Liang et al., 2013)
miR-765	ovarian cancer	decrease VEGF-A and AKT/SRC signaling	n/a	suppress	(Salinas-Vera et al., 2019)
miR-200a	ovarian cancer	EphA2 targeting	n/a	suppress	(Sun et al., 2014)
miR-141	glioma				(Li, Huang, et al., 2018a)
miR-26b	glioma		n/a		(Wu et al., 2011)
			n/a		
miR-27b	ovarian cancer	VE-cadherin targeting	n/a	suppress	(Liu et al., 2017)
miR-27a-3p	HCC		n/a		(Zhao, Sun, et al., 2016a)
miR-450a-5p	colorectal cancer	suppress SOX2 expression	n/a	suppress	(Chen et al., 2020)
miR-101	HCC	target TGF-ßR1 and Smad2 in tumor cells, and reduce SDF1 levels in CAFs	n/a	suppress	(Yang et al., 2016)
miR-186	prostate cancer	Twist1 targeting	n/a	suppress	(Zhao et al., 2016b)
miR-Let-7f	glioma	periostin targeting	n/a	suppress	(Xue et al., 2016)
miR-9	glioma	stathmin 1 targeting	n/a	suppress	(Song et al., 2013)
miR-584-3p	glioma	ROCK1 targeting	n/a	suppress	(Xu et al., 2017)
LncRNAs:					
TP73-AS1	TNBC	release the miR-490-3p's post-translational repression on Twist1	miR-490-3p	promote	(Tao et al., 2018)
LINC00312	lung adenocarcinoma	YBX1 as the interacting partner to increase VE-cadherin, TGF-β, VEGF-A/-C levels *via* AKT	unknown	promote	(Peng et al., 2018)
MALAT1	non-small cell lung cancer	upregulated upon ERβ activation, prevent NEDD9 repression	miR-145-5p	promote	(Yu et al., 2019)
	gastric cancer	ERK/MMP and FAK/paxillin signaling	miR-376a		(Li, Wu, et al., 2017b)
n339260	HCC	linked to c-Myc/SOX2 overexpression; increase VE-cadherin levels	miR-31-3p, miR-30e-5p, miR-520c-5p, miR-519c-5p, miR-29b-1-5p	promote	(Zhao et al., 2018)
LINC00339	glioma	activate Twist1/MMP-2/MMP-14 signaling	miR-539-5p	promote	(Guo et al., 2018)
HOXA-AS2	glioma	downstream EGFR signaling, increase VE-cadherin and MMP-2/−9 levels	miR-373	promote	(Gao et al., 2018)
ZNRD1-AS1	glioma	regulate ELF1/EMI1 axis	miR-499a-5p	promote	(Wang, Song, et al., 2020a, Wang, Yang, et al., 2020b)
SNHG20	glioma	increase SNHG20 stability, promote FOXK1 degradation and increase MMP-1/−9 and VE-cadherin levels	unknown	promote	(Li et al., 2019)
HCG15	glioma	ZNF331 targeting	unknown	promote	(Jing et al., 2020)

Table 2
Summary of therapeutics targeting vasculogenic mimicry.

Molecules	Experimental model	References
HDAC inhibitors		
Vorinostat Trichostatin A Entinostat (MS275) MC1568	U87MG cells & GSCs	(Pastorino et al., 2019)
Entinostat (MS275)	Triple-negative breast cancer cells (MDA-MB-231, LM2-4,BT-549, MCF-7 cells)	(Maiti et al., 2019)
CSCinhibitors		
Salinomycin	CSCs	(Gupta et al., 2009)
Tyrosine kinase inhibitors		
Anti-VEGFR1-mAb (D16F7)	Melanoma & glioblastoma	(Graziani et al., 2016); (Atzori et al., 2017)
Sorafenib	HCC patients	(Mao et al., 2020)
MMP inhibitors		
Incyclinide derivative (COL-3)	Metastatic cancers	NCT00001683
Doxycycline	NCI-H446, A549 cells & HCC	(Ko et al., 2015); (Cao et al., 2013); (Meng et al., 2014)
TGF-βR1 antagonist		
Galunisertib	Glioma	(Zhang et al., 2016)
Molecules from plants:		
Brucine	MDA-MB-231 cells	(Xu et al., 2019)
Polyphyllin I	HCC	(Xiao et al., 2018)
Lupeol	B16F10 cells	(Bhattacharyya et al., 2019)