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Cancer and Blood Vessels: A Complex Relationship

Francesco Pezzella*

Nuffield Division of Clinical Laboratory Science, Radcliffe Department of Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK

***Corresponding author:** Pezzella F, Nuffield Division of Clinical Laboratory Science, Radcliffe Department of Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK, Tel: +44 01865 220497; **E-mail:** francesco.pezzella@ndcls.ox.ac.uk

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Abstract

The relationship between vessels and cancer has been the subject of many studies. In 1787 was formulated the idea that tumors always induced growth of new vessels, a phenomenon called "angiogenesis". The growth of new capillaries into tumor implants was formally described in animal models only in 1939 but during the same years was also published that only pre-existing vessels could be identified inside some neoplastic lesions. During the 1970s, Folkman started to systematically investigate the role of newly formed vessels in cancer and the angiogenesis theory, i.e. the complete reliance of cancers on new vessels in order to grow, become predominant. Eventually recent work has demonstrated that some tumors can truly grow without inducing angiogenesis as the neoplastic cells exploit the preexisting vessels by co-opting them.

Keywords Cancer; Blood vessels; Angiogenesis

Introduction

The human body has a very rich and intricate network of blood vessels; therefore it is not surprising that the relationship between vessels and cancer has been the subject of many studies. In 1787 [1] was formulated the idea that, despite the presence of the normal vascular system, tumors always induced growth of new vessels, a phenomenon called "angiogenesis". The growth of new capillaries into tumor implants was formally described in animal models only in 1939

[2] but during the same years was also published that only pre-existing vessels could be identified inside some neoplastic lesions [3,4]. Eventually, during the 1970s, Folkman [5] started to systematically investigate the role of newly formed vessels in cancer and the angiogenesis theory, i.e. the complete reliance of cancers on new vessels in order to grow, become predominant.

Classic Tumor Angiogenesis

What is the evidence that tumors are angiogenesis dependent [6]. In 1971 Folkman formally enunciated his concept that "the growth of solid neoplasm is always accompanied by neo-vascularization" [7]. He was relying mostly on "in vitro" and animal models [6] in which experiments had been frequently conducted in anatomical regions containing little or no vessels at all, like the cornea of a rabbit. As matter of fact most of the research leading to the angiogenesis theory has been carried out animal models [8] with fewer studies done on actual tumor samples and a histopathological study, classifying intra-tumor vessels, maintained that they were all newly formed [9].

A second hypothesis followed, that the higher the density of new micro-vessels the higher the aggressiveness of the tumor and the worst the outcome for the patient [10,11]. A series of meta-analysis has subsequently challenged this hypothesis [12-14] with some studies actually showing an altogether different picture, with higher micro vessel density predicting a better outcome [15,16]. Nevertheless, "inducing angiogenesis" is still regarded as a hallmark of cancer [17].

The mechanisms of classic angiogenesis

Vessels are usually quiescent and their homeostasis is controlled mainly by the Angiopoietin family (ANG1, ANG2 and ANG4) and their receptors (TIE-1 and TIE-2) [18].

If growth of new vessels is required, the main pathway involved is the VEGF one. There are four VEGF proteins, A, B, C and D and three receptors, VEGF1R/flt-1, VEGFR2/kdr and VEGFR3/flt4. The VGFA isoforms 121 and 165 plus the VGFR2, a type III receptor tyrosine kinase role are regarded as the most important. Another membrane protein, neuropillin, has been shown to link to VEGFA165 and to produce an angiogenic effect similar to that observed following the activation of VEGFR2 [19]. As a tumour starts to outgrow its blood supply some of its areas become hypoxic inducing the secretion of angiogenic factors, consequently endothelial cells of the vessels already present in the lesion loosen their junctions and detach from the basal membrane through the action of metalloproteases (MMPs). Following the continuous increases of hypoxia and therefore of VEGFA levels, the vessels become leaky [20] as more endothelial cells break through the basal membrane and start to migrate alongside the gradient of angiogenc proteins and begin to secrete ANG2, which competes with ANG1 for the TIE2 receptor, further inducing endothelial cell detachment, vascular permeability and endothelial proliferation [18].

The docking of VEGFA to VEGFR2 triggers also the activation of the PLC (Phospholipase C) gamma/PKC/RAF MEK/MAPK pathway, leading to the proliferation of the endothelium, and of the PI3K/AKT pathway, resulting into its increased survival [19]. After crossing the basal membrane, the endothelial cells proliferate and accumulate forming a cord of cells: the one closer to the vessel of origin are the Stalk cells while the most advanced toward the angiogenic signal are the Tip cells which lead the migration. The differentiation into either Stalk cells or Tip cells is regulated by VEGFA throughout the NOTCH/WNT pathway. NOTCH (1,2,3 and 4) are receptors expressed

on different cell types, their ligand (Jag 1 and 2 plus Dl13 and 4) are also present on the cell membrane.

All the endothelial cells express both NOTCHs and NOTCH ligands (Jag 1,2 Dll1,3 and 4). Following VEGFR2 activation by VEGFA the endothelial cells will start to increase the transcription of DLL4. The Tip cells, which are the closest to the hypoxic area along the endothelial sprout and are subject to a higher VEGFA concentration, will accumulate more DLL4. Compared to the stalk cells the Tips cell will therefore have a higher DLL4: NOTCH ratio and subsequently, higher levels of DLL4 linked to the extracellular domain of NOTCH will be imported in the cytoplasm leaving behind a fragment of NOTCH. The NOTCH: DLL4 complex is not a transcription factor, the transcription factor activity lies with the residual intracellular fragment of NOTCH left behind which is also detached from the membrane and is the one that will trigger the transcription of the downstream pathways which will than reduce the proliferative effect of VEGF, contributing at the maturation process of the newly formed vessel.

Therefore the most advanced endothelial Tip cells, having more DLL4, will acquire a phenotype characterized by low proliferation, lack of vascular lumen formation but VEGFA dependent production of filopodia rich in actin, resulting in increased motility. The Stalk cells will have instead a phenotype characterized by low motility and higher proliferation which is however progressively switched of, as NOTCH activation increases, leading to vascular lumen formation, establishment of inter endothelial junctions and deposition of basal membrane. As a lumen is established, the arrival of oxygenated blood start to inhibits VEGFA further decreasing the proliferation. Eventually new pericytes are recruited and the maturation of the vessel is completed [21].

During the angiogenic process therefore there will be the sprouting of a new vessels formed by endothelial cells with a "gradient of phenotype": highly mobile but not proliferating at the tip, progressively less mobile going down along the new vessels, and with higher proliferation in the middle section but re-establishment of lumen, oxygenation and quiescence at the beginning. Eventually the newly formed branches will fuse with other vessels and the all the new vessels, well oxygenated, will acquire a quiescent phenotype.

Bone marrow-derived endothelial progenitor cells

During tumour angiogenesis however the Endothelial Cells (EC) involved can also be recruited from circulating Endothelial Progenitor Cells (EPC) [22]. These cells are mainly found in the bone marrow but can also be isolated from peripheral blood, fetal liver and umbilical cord blood [23], their phenotype is variable according to the organ of provenience but they always express CD34 and VEGFR2 [24]. When a tumor secrets cytokines and hypoxia related proteins attract EPC to the neoplastic formation where it is assumed that they can play two main roles: further increases the angiogenic stimulus by secreting pro-angiogenic factors and to be recruited inside the newly establish vascular structure where the EPC differentiated into mature endothelial cells [24].

The Non-angiogenic Tumours

Human pathology: The description of completely non-angiogenic tumors was firstly done by looking at pattern of vascularization in non-Small Cell Lung Carcinomas (nSCLC) [25-28], where it was also observed that in many neoplasm both angiogenic and non-angiogenic areas co-exist [26]. Quite surprisingly it was also found that these malignancies were actually more aggressive than the angiogenic ones [29,30]. Furthermore non-angiogenic metastases to the lung, originating form an angiogenic primary [31], were identified [25,30] questioning the idea that angiogenesis is linked to tumor progression and rather suggesting that angiogenesis can be switched off during the spreading of a tumor. The absence of newly formed vessels and the persistence of the preexisting lung vasculature has been confirmed by immunohistochemical studies looking at the expression of LH39 and aVb3 which demonstrated that the vessels present in the non-angiogenic tumors have the same phenotype of the normal lung [32]. A three dimensional reconstruction of normal tissue and lung carcinomas confirmed that the vascular infrastructure of the non-angiogenic tumors was indistinguishable from that of the normal tissue [33]. This type of non-angiogenic growth of neoplastic cells inside the lung has been in the past reported as "intra alveolar" [3], the very first description going back as far as to 1861 [34].

As the human body is very rich in vessels, we expected to find non-angiogenic tumors also in other organ. Vermeulen et al. [35,36] described three different ways in which breast and colorectal metastatic malignancy can grow in relationship to the normal vasculature of the liver. In two patterns there was destruction of the liver structure with angiogenesis inside the tumor. In the third pattern, the replacement pattern, the metastatic cancer cells substitute the hepatocytes leaving the overall preexisting hepatic architecture intact. No fibrosis is seen, inflammation is almost absent and tumor cells and hepatocytes have intimate cell-cell contact and the tumor cells are growing by co-opting the sinusoidal blood vessels. As in the lung, also the liver metastatic lesions can have a pure or mixed pattern of growth. Sometime is a single metastasis containing both angiogenic and non-angiogenic areas, sometime, in patients with multiple metastases, some lesions are purely angiogenic and some are purely non-angiogenic [37]. The patients with non-angiogenic metastases have slightly better chance of survival after 24 months but not after 60 months. According to the organ of origin (breast, pancreatic and urinary bladder) the prevalence non-angiogenic growth pattern is different [38], only one third of the colorectal [39] secondary grows in this way in the liver but almost all the breast cancer metastases are non-angiogenic. There is also a second non-angiogenic growth pattern, in which the cancer cells spread within the hepatic sinusoids rather than replace the hepatocytes [40].

In 1994 a hypothesis was raised that also glioblastoma multiforme could growth by exploiting preexisting vessels [41]. Since then the occurrence of vascular co-optation in brain has been confirmed for glioblastoma [42] and reported also for glioma [43,44]. Not only primary tumors but brain metastases as well can growth by vascular co-optation [45].

Animal models

Non-angiogenic growth by vascular co-optation has by now also been described in animal models. The first report was in a rat model [46] in which glioma and mammary neoplastic cells implanted in the brain could briefly growth along pre-existing vessels leading to the conclusion that a significant, although only transient, non-angiogenic neoplastic growth was occurring. Subsequently other studies confirmed not only that malignant cells can co-opt vessels in mice

brain but also that full non-angiogenic tumours can grow [43,47] in lung [48] and liver [49].

Why tumours grow in a non-angiogenic way?

In order to unravel the mechanisms leading to neoplastic growth by vascular co-optation in primary non-small cell lung carcinoma we started by investigating some basic characteristics. No major differences were observed as far as necrosis, apoptosis and hypoxia is concerned while the angiogenic tumours contained more inflammation, fibrosis and expression of thrombospondin in the stroma [50,51]. mRNA expression profiling confirmed that no differences in classic hypoxia/angiogenesis pathways could be found with the exception of Thrombospondin1 [52].

Surprisingly instead we found, in non-angiogenic tumors an increased transcription of a set of genes linked to Oxidative Phosphorylation, suggesting the possibility of an undergoing metabolic reprogramming. The second major finding was the decreased level, in the same tumors, of mRNA transcripts of several adhesion molecule, leading us to raise the hypothesis that diminished cell to cell contact could be associated with failure to develop a vascular infrastructure [52]. A third finding was the higher rate of cytoplasm p53 expression in non-angiogenic malignancies and in a small pilot study we also found a higher incidence of p53 mutations in these lesions [51]. If confirmed in larger studies these data would be consistent with the report that, in animal models, inactivation of p53 is associated with resistance to anti-angiogenic drugs, as it increases their ability to survive in hypoxia [53].

Less data are available as far as the biological characterization of the non-angiogenic liver metastases is concerned. However the different type of spreading compared to the lung (replacement of normal cells versus filling empty spaces) and the finding that very scanty CA9 expression is present at the edge of the replacement pattern indicate that these metastases are not very hypoxic [38] and suggests that liver and lung non-angiogenic tumours could have different underlying mechanisms

Vascular co-option: molecular mechanisms

Cancer can grow without angiogenesis because malignant cells can exploit pre-existing vessels by co-opting them. Only a few studies are so far available on the mechanism of vascular co-option and they are mostly focused on co-option of the brain vessels.

The first question is how the neoplastic cells are attracted towards the vessel. In a work on gliomas Montana et al. [54] show that this attraction is mediated by bradykinin signalling pathways: the glioma cell expresses Bradykinin 2 Receptor (B2R), which is activated by the bradykinin secreted by the endothelial cells. Once activated B2R induces intracellular Ca2+ oscillations triggering the migration of the neoplastic cells toward the vessel along the gradient of secreted bradykinin.

In a second study Caspani et al. [55] unravelled the interaction mechanism between Glioblastoma Multiforme (GBM) cells and the pericytes of the brain vessels, both in animals and in vitro models. They describe that GMB cells produce cytoplasm extensions, denominated flectopodia which relay on CDC42, a GTPAse that regulates these actions-dependent cytoplasmic extensions. These flectopodia adhere to the pericytes through the adhesion molecule CD44. Following the co-option, the pericytes contract, inducing changes of the vascular structure from linear to convoluted. In in-vitro experiments fusion between the cytoplasm of the GMB cells and those of the pericytes follows the interaction between the two types of cells resulting in the formation of hybrid cells. Inhibition of CDC42 results in impaired vascular co-option and differentiation of some pericytes into macrophage-like cells with anti-tumour activity. The possibility that this process could be actually occurring in humans is supported by immunohistochemical staining demonstrating increased levels of CDC42 and CD44 expression in perivascular GBM cells on GBM histological sections.

Different are the requirement for the metastatic cells as they reach the brain by the blood vessels. Valiente et al. [56] demonstrated that metastatic cells resist apoptosis and co-opt brain vessels by expressing the protein neuroserpin, an inhibitor of plasmin. Plasmin promotes the apoptosis of cancer cells and inhibits their spread along the vasculature a defensive reaction of the brain tissue to malignant cells entering the parenchyma. Linking these two events (the entrance of metastatic tumour cells and the production of plasmin) is the astrocyte which, in response to inflammation, parenchyma damage and the passage of metastatic cells across the brain barrier, expresses high levels of two proteins: Fas ligand (FasL) and Plasminogen Activator (PA). Increased levels of PA lead to cleavage of plasminogen (which is secreted by neurons) into the active form, plasmin, which can then act on several other proteins, including FasL and the adhesion molecule L1CAM.

FasL is bound to the membrane of astrocytes, but plasmin is able to cleave and release it as soluble form, sFasL, which in turn can link to its receptor, Fas, on the tumour cell and trigger apoptosis. When cancer cells express high level of neuroserpin, the astrocytes are prevented from expressing PA, consequently the release of plasmin from plasminogen is stopped, secretion of sFasL is blocked and thus is apoptosis of the tumor cell.

Once the survival of the metastatic cells is achieved how they will adhere to the co-opted vessels? One mechanism is again linked to neuroserpin expression: by inhibiting generation of plasmin, the L1CAM molecule expressed by the tumor cell is not cleaved and remains intact allowing the metastatic cells to adhere to the outbound surface of the vessels [56].

In models of metastatic breast cancer and melanoma vascular co-option in vivo and cancer cell-endothelium adhesion in vitro are reported to be dependent upon the induction of Connexin 26 (Cx26) and Connexin 43 (Cx43) expression by the twist transcription factor. These two connexins induce the formation of functional Gap Junctions between cancer cells and endothelium. Silencing of these connexins in both breast adenocarcinoma and melanoma cells lose the ability, not only to adhere at the endothelial cells in vitro but also to co-opt vessels in vivo in zebra fish, chicken embryo and mouse models [57]. Presence of beta1 integrin is instead mediating the adhesion between neoplastic cells and the vascular basal membrane [58].

The effect of co-option on the blood vessels

In rat models of glioma Holash et al. [46] have observed vascular co-option, but only as a transient phenomenon in the early stages of tumour growth, before the triggering of angiogenesis. Adult mouse blood vessels express high levels of Angiopoietin 1 which antagonises Angiopoietin 2 on the Tie2 receptor, and maintain the mature vessel stable by inducing anchorage to the basal membrane and protecting form apoptosis. The first event observed following co-option was an increase in the levels of Angiopoietin 2 in the preexisting vessels surrounded by tumor cells. Higher levels of Angiopoietin 2, without increases of VEGF expression, induced vascular regression by detachment of the endothelium from the basal membrane and

subsequent apoptosis. However in this model, VEGF expression was induced only on the hedge of the tumour triggering neo-angiogenesis. It is therefore likely that a different response occurs in long-term non-angiogenic neoplasia.

Vasculogenic Mimicry

Alongside vascular co-optation, Vasculogenic Mimicry (VM), the ability of tumor cells to form vessel-like networks, is another alternative way for tumors to receive oxygen and nutrients without inducing angiogenesis [59]. Firstly described in melanoma, it has now been reported also in several other types of tumors [60,61]. It differs from vascular co-optation because functional channels are created but they are not made up by newly formed vessels but by the very same tumor cells. VM appears to recapitulate the formation of the vasculogenesis network, as in these aggressive tumor neoplastic cells reverse to an embryonic-like phenotype, therefore becoming able to mimic endothelial cells [59].

The main signaling pathway regulating VM is ignited by up-regulation of VE-cadherin by Hif 2. VE-cadherin co-localize with phosphorylates, EphA2 which in turn activate PI3K both directly and throughout FAK/ERK1/2. PIK3 induce the cleavage of pro-MT1-MMP and pro-MMP2 into their corresponding active forms which cleave laminin5γ2 realizing the pro-migratory fragments 5γ2′ and 5γ2x. These two latter protein fragments induce and guide the arrangement of the neoplastic cells into channel structures. This pathway can be inhibited by cAMP, blocking ERK1/2 function. However cAMP can also sustain VM by positively regulating VE-Cadherin expression throughout the Nodal/Notch 1/4 pathway with Gal-3 having a similar positive effect on VM [60,62].

Blood Vessels and Anti-angiogenic Treatment

As we predicted [26] vascular co-option is now recognized as one of the causes for resistance to anti-angiogenic treatment [19]. Other reasons for resistance include the heterogeneous composition of the newly formed intra-tumor vessels. The latter especially applies to spontaneous tumors that can develop over a considerable period of time, as opposed to rapidly growing experimental cancers. Dvorak et al. have demonstrated the existence of such vascular heterogeneity with some of these vessels growing independently form VEGF [63]. Furthermore anti-angiogenic treatments may lead to even more heterogeneity [64]. Vascular biology can also change during metastatic progression and this may explain why lung metastases can be resistant to sunitinib while the same tumor cell line is sensitive to this drug as primary tumor [65].

The characterization of tumors, according to their angiogenic, or non-angiogenic phenotype helps therefore to explain why some tumors resist to anti angiogenic treatment. Consequently will also help to identify those tumors that can be selectively targeted for anti angiogenic treatment as they are truly relying on new vessels formation. It is therefore possible that by this approach we will be able to obtain better results from anti-angiogenic drugs. Furthermore a better understanding of the biology of non-angiogenic tumors and of how they co-opt vessels could results in the discovery of novel treatment targets.

Conclusions

"Inducing angiogenesis" a hallmark too far? It is now established that tumors can also develop and progress in absence of angiogenesis. The biological implication of the new data obtained so far is that the development of hypoxia in a neoplastic lesion does not always trigger angiogenesis. While some tumors appear to be completely non-angiogenic tumors, many others contain both angiogenic and non-angiogenic areas. This is in disagreement with the theory of Folkman [6], regarded as one of Hallmark of Cancer [17], stating that that tumours must induce angiogenesis to grow.

References

1. Hall AP (2005) The role of angiogenesis in cancer. Comparative Clinical Pathology 13: 95-99.
2. Ide AG, Baker NH, Warren SL (1939) Vascularization of the Brown Pearce rabbit epithelioma transplant as seen in the transparent ear chamber. American Journal of Roentgenology 42: 891-899.
3. Willis RA (1934) The spread of tumours in the human body. London: Churchill BJS 22: 196.
4. Ritchie AC (1962) The classification, morphology, and behaviour of tumours. In: Florey H, ed. General Pathology. Third Edition ed. London: Lloyd-Luke (Medical Books) LTD 1962: 551-597.
5. Zetter BR (2008) The scientific contributions of M. Judah Folkman to cancer research. Nat Rev Cancer 8: 647-654.
6. Folkman J (1990) What is the evidence that tumors are angiogenesis dependent? J Natl Cancer Inst 82: 4-6.
7. Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285: 1182-1186.
8. Hanahan D, Folkman J (1996) Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 86: 353-364.
9. Nagy JA, Chang SH, Shih SC, Dvorak AM, Dvorak HF (2010) Heterogeneity of the tumor vasculature. Semin Thromb Hemost 36: 321-331.
10. Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, et al. (1992) Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. Journal of the National Cancer Institute 84: 1875-1887.
11. Brawer MK (1996) Quantitative microvessel density. A staging and prognostic marker for human prostatic carcinoma. Cancer 78: 345-349.
12. Trivella M, Pezzella F, Pastorino U, Harris AL, Altman DG; Prognosis In Lung Cancer (PILC) Collaborative Study Group (2007) Microvessel density as a prognostic factor in non-small-cell lung carcinoma: a meta-analysis of individual patient data. Lancet Oncol 8: 488-499.
13. Yu M, Liu L, Liang C, Li P, Ma X, et al. (2014) Intratumoral vessel density as prognostic factors in head and neck squamous cell carcinoma: a meta-analysis of literature. Head Neck 36: 596-602.
14. Pastushenko I, Vermeulen PB, Carapeto FJ, Van den Eynden G, Rutten A, et al. (2014) Blood microvessel density, lymphatic microvessel density and lymphatic invasion in predicting melanoma metastases: systematic review and meta-analysis. The British journal of dermatology 170: 66-77.
15. Couvelard A, O'Toole D, Turley H, Leek R, Sauvanet A, et al. (2005) Microvascular density and hypoxia-inducible factor pathway in pancreatic endocrine tumours: negative correlation of microvascular density and VEGF expression with tumour progression. British journal of cancer 92: 94-101.
16. Prall F, Gringmuth U, Nizze H, Barten M (2003) Microvessel densities and microvascular architecture in colorectal carcinomas and their liver metastases: significant correlation of high microvessel densities with better survival. Histopathology 42: 482-491.
17. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144: 646-674.
18. Augustin HG, Koh GY, Thurston G, Alitalo K (2009) Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. Nat Rev Mol Cell Biol 10: 165-177.
19. Kerbel RS (2008) Tumor angiogenesis. N Engl J Med 358: 2039-2049.

20. Dvorak HF, Brown LF, Detmar M, Dvorak AM (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. 146: 1029-1039.
21. Phng LK, Gerhardt H (2009) Angiogenesis: a team effort coordinated by notch. Dev Cell 16: 196-208.
22. Nolan DJ, Ciarrocchi A, Mellick AS, Jaggi JS, Bambino K, et al. (2007) Bone marrow-derived endothelial progenitor cells are a major determinant of nascent tumor neovascularization. Genes Dev 21: 1546-1558.
23. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, et al. (2000) Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 95: 952-958.
24. de la Puente P, Muz B, Azab F, Azab AK (2013) Cell trafficking of endothelial progenitor cells in tumor progression. Clinical cancer research: an official journal of the American Association for Cancer Research 19: 3360-3368.
25. Pezzella F, Di Bacco A, Andreola S, Nicholson AG, Pastorino U, et al. (1996) Angiogenesis in primary lung cancer and lung secondaries. Eur J Cancer 32A: 2494-2500.
26. Pezzella F, Pastorino U, Tagliabue E, Andreola S, Sozzi G, et al. (1997) Non-small-cell lung carcinoma tumor growth without morphological evidence of neo-angiogenesis. Am J Pathol 151: 1417-1423.
27. Offersen BV, Pfeiffer P, Hamilton-Dutoit S, Overgaard J (2001) Patterns of angiogenesis in nonsmall-cell lung carcinoma. Cancer 91: 1500-1509.
28. Sardari Nia P, Colpaert C, Vermeulen P, Weyler J, Pezzella F, et al. (2008) Different growth patterns of non-small cell lung cancer represent distinct biologic subtypes. Ann Thorac Surg 85: 395-405.
29. Pastorino U, Andreola S, Tagliabue E, Pezzella F, Incarbone M, et al. (1997) Immunocytochemical markers in stage I lung cancer: relevance to prognosis. J Clin Oncol 15: 2858-2865.
30. Nia PS, Colpaert C, Blyweert B, Kui B, Vermeulen P, et al. (2004) Prognostic value of nonangiogenic and angiogenic growth patterns in non-small-cell lung cancer. Br J Cancer 91: 1293-1300.
31. No authors listed (2000) Evidence for novel non-angiogenic pathway in breast-cancer metastasis. Breast Cancer Progression Working Party. Lancet 355: 1787-1788.
32. Passalidou E, Trivella M, Singh N, Ferguson M, Hu J, et al. (2002) Vascular phenotype in angiogenic and non-angiogenic lung non-small cell carcinomas. Br J Cancer 86: 244-249.
33. Adighibe O, Micklem K, Campo L, Ferguson M, Harris A, et al. (2006) Is nonangiogenesis a novel pathway for cancer progression? A study using 3-dimensional tumour reconstructions. British journal of cancer 94: 1176-1179.
34. Erichsen J (1861) Zwei falle von carcinosis acuta miliaris. Virchows Archiv 21: 465-479.
35. Vermeulen PB, Colpaert C, Salgado R, Royers R, Hellemans H, et al. (2001) Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. The Journal of pathology 195: 336-342.
36. Stessels F, Van den Eynden G, Van der Auwera I, Salgado R, Van den Heuvel E, et al. (2004) Breast adenocarcinoma liver metastases, in contrast to colorectal cancer liver metastases, display a non-angiogenic growth pattern that preserves the stroma and lacks hypoxia. British journal of cancer 90: 1429-1436.
37. Eefsen RL, Van den Eynden GG, Hoyer-Hansen G, Brodt P, Laerum OD, et al. (2012) Histopathological growth pattern, proteolysis and angiogenesis in chemonaive patients resected for multiple colorectal liver metastases. Journal of oncology 2012: 907971.
38. Van den Eynden GG, Majeed AW, Illemann M, Vermeulen PB, Bird NC, et al. (2013) The multifaceted role of the microenvironment in liver metastasis: biology and clinical implications. Cancer Res 73: 2031-2043.
39. Van den Eynden GG, Bird NC, Majeed AW, Van Laere S, Dirix LY, et al. (2012) The histological growth pattern of colorectal cancer liver metastases has prognostic value. Clinical & experimental metastasis 29: 541-549.
40. Augustin HG, Koh GY, Thurston G, Alitalo K (2009) Control of vascular morphogenesis and homeostasis through the angiopoietin-Tie system. Nat Rev Mol Cell Biol 10: 165-177.
41. Kerbel RS (2008) Tumor angiogenesis. N Engl J Med 358: 2039-2049.
42. Dvorak HF, Brown LF, Detmar M, Dvorak AM (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. 146: 1029-1039.
43. Phng LK, Gerhardt H (2009) Angiogenesis: a team effort coordinated by notch. Dev Cell 16: 196-208.
44. Nolan DJ, Ciarrocchi A, Mellick AS, Jaggi JS, Bambino K, et al. (2007) Bone marrow-derived endothelial progenitor cells are a major determinant of nascent tumor neovascularization. Genes Dev 21: 1546-1558.
45. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, et al. (2000) Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 95: 952-958.
46. de la Puente P, Muz B, Azab F, Azab AK (2013) Cell trafficking of endothelial progenitor cells in tumor progression. Clinical cancer research: an official journal of the American Association for Cancer Research 19: 3360-3368.
47. Pezzella F, Di Bacco A, Andreola S, Nicholson AG, Pastorino U, et al. (1996) Angiogenesis in primary lung cancer and lung secondaries. Eur J Cancer 32A: 2494-2500.
48. Pezzella F, Pastorino U, Tagliabue E, Andreola S, Sozzi G, et al. (1997) Non-small-cell lung carcinoma tumor growth without morphological evidence of neo-angiogenesis. Am J Pathol 151: 1417-1423.
49. Offersen BV, Pfeiffer P, Hamilton-Dutoit S, Overgaard J (2001) Patterns of angiogenesis in nonsmall-cell lung carcinoma. Cancer 91: 1500-1509.
50. Sardari Nia P, Colpaert C, Vermeulen P, Weyler J, Pezzella F, et al. (2008) Different growth patterns of non-small cell lung cancer represent distinct biologic subtypes. Ann Thorac Surg 85: 395-405.
51. Pastorino U, Andreola S, Tagliabue E, Pezzella F, Incarbone M, et al. (1997) Immunocytochemical markers in stage I lung cancer: relevance to prognosis. J Clin Oncol 15: 2858-2865.
52. Nia PS, Colpaert C, Blyweert B, Kui B, Vermeulen P, et al. (2004) Prognostic value of nonangiogenic and angiogenic growth patterns in non-small-cell lung cancer. Br J Cancer 91: 1293-1300.
53. No authors listed (2000) Evidence for novel non-angiogenic pathway in breast-cancer metastasis. Breast Cancer Progression Working Party. Lancet 355: 1787-1788.
54. Passalidou E, Trivella M, Singh N, Ferguson M, Hu J, et al. (2002) Vascular phenotype in angiogenic and non-angiogenic lung non-small cell carcinomas. Br J Cancer 86: 244-249.
55. Adighibe O, Micklem K, Campo L, Ferguson M, Harris A, et al. (2006) Is nonangiogenesis a novel pathway for cancer progression? A study using 3-dimensional tumour reconstructions. British journal of cancer 94: 1176-1179.
56. Erichsen J (1861) Zwei falle von carcinosis acuta miliaris. Virchows Archiv 21: 465-479.
57. Vermeulen PB, Colpaert C, Salgado R, Royers R, Hellemans H, et al. (2001) Liver metastases from colorectal adenocarcinomas grow in three patterns with different angiogenesis and desmoplasia. The Journal of pathology 195: 336-342.
58. Stessels F, Van den Eynden G, Van der Auwera I, Salgado R, Van den Heuvel E, et al. (2004) Breast adenocarcinoma liver metastases, in contrast to colorectal cancer liver metastases, display a non-angiogenic growth pattern that preserves the stroma and lacks hypoxia. British journal of cancer 90: 1429-1436.
59. Eefsen RL, Van den Eynden GG, Hoyer-Hansen G, Brodt P, Laerum OD, et al. (2012) Histopathological growth pattern, proteolysis and angiogenesis in chemonaive patients resected for multiple colorectal liver metastases. Journal of oncology 2012: 907971.
60. Van den Eynden GG, Majeed AW, Illemann M, Vermeulen PB, Bird NC, et al. (2013) The multifaceted role of the microenvironment in liver metastasis: biology and clinical implications. Cancer Res 73: 2031-2043.

61. Van den Eynden GG, Bird NC, Majeed AW, Van Laere S, Dirix LY, et al. (2012) The histological growth pattern of colorectal cancer liver metastases has prognostic value. Clinical & experimental metastasis 29: 541-549.

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