Chinese-German J Clin Oncol
DOI 10.1007/s10330-014-1304-5
May 2014, Vol. 13, No. 5, P207–P211

Vasculogenic mimicry in non-small cell lung cancer and its relationship with tumor stage

Xiangqian Lu, Xiao Li, Fangzhen Shen, Wenjing Xiao

Department of Oncology, The Affiliated Hospital of Qingdao University, Qingdao 266003, China

Received: 11 March 2014 / Revised: 28 March 2014 / Accepted: 18 April 2014

Abstract ***Objective:*** The purpose of the study was to study the mechanism of vasculogenic mimicry (VM) and its relationship with tumor stage in non-small cell lung cancer (NSCLC). ***Methods:*** Forty-two patients with NSCLC were collected, 19 belonged to the early stage (stages I + II) while 23 were late stage (stages III + IV). Moreover, 20 patients got surgical treatment and 22 got chemotherapy. We studied the relationship of VM with stage, chemotherapeutic effect, HIF-1α, microvessel density (MVD) and clinicopathologic features. ***Results:*** VM in patients of early stages were significantly more than late stages (68.4% vs 26.1%, $P = 0.006$), and the positive rate of VM was proportional to HIF-1α ($P = 0.034$). But no correlation was found between VM and chemotherapeutic effect (14.3% vs 26.7%, $P = 1.00$) or MVD ($P > 0.05$). Furthermore, we found VM also showed a negative correlation with distant metastases and lymph nodes metastases ($P < 0.05$) while no correlation was found with other clinicopathologic. ***Conclusion:*** VM was generated during the early stage in NSCLC and correlated with lymph nodes metastases. As the disease progressed, VM may be replaced by vascular endothelial cells, so the late-stage patients especially people with distant metastases had fewer VM. As the main factor produced by hypoxia, HIF-1α may make a difference in VM formation. Thus we inferred VM might be a new target for targeted therapy, and could provide help for clinical staging and treatment.

Key words vasculogenic mimicry (VM); angiogenesis; non-small cell lung cancer (NSCLC); targeted therapy; microvessel density (MVD)

Lung cancer has been the most common of death by malignancy in some countries. The 85% of lung cancers were non-small cell lung cancers (NSCLCs). Only minority of the patients can be diagnosed and treated surgically, whose 5 year survival rate may reach a length of nearly 50%. However, most of the patients belong to the advanced stage when diagnosed that have no chance to get the surgical treatment. Like other tumors, the growth of lung cancer depends on new angiogenesis when its size is bigger than 2–3 mm, raised by Folkman in 1971 [1]. Subsequently, researchers demonstrated the angiogenesis theory, and found some factors were closely related to its regulation, for instance, VEGF, bFGF, HIF-1α [2]. Microvessel density (MVD) means the average microvessels' number in unit tissue area, and was the quantitative indicator of angiogenesis [3].

In 1999, Maniotis found a structure when studying human eyes uveal melanoma microcirculation, through which red blood cells passed and that can be stained positively in the absence of epithelial cell markers, named vasculogenic mimicry (VM) [4]. Thus far, angiogenesis mainly includes two patterns – endothelial cells dependent angiogenesis and non-endothelial cells dependent angiogenesis. Subsequent studies demonstrated the existence of VM in many other malignancies [5–9], while no VM was proved in benign lesions and tumors. Investigators [10–12] reported the discovery of VM in NSCLC. Some investigators put forward the three-stage theory, which supported that angiogenesis experienced three stages, in order, VM, MV (mosaic vessel) and endothelial cells dependent vessel, as the endothelial cells moving inward and coverage along the channel [13, 14]. However, though many experiments have been done, the VM regulatory mechanism is still unknown. Investigations found hypoxia played an significant role in angiogenesis besides VM formation, in which HIF-1α was a key factor [15, 16].

This paper is aimed at preliminarily discussing the mechanism of VM in NSCLC, and demonstrating whether the chemosensitivity of patients with NSCLC has correlation with VM, thus we inferred to provide help for clinical staging and treatment.

Correspondence to: Fangzhen Shen. Email: shenfangzhen@163.com

Materials and methods

Patients

A total of 42 patients with NSCLC diagnosed in the Affiliated Hospital of Qingdao University (China) were enrolled in the study. Getting their surgical or percutaneous lung biopsy sections. Details of patients were shown in Table 1. We divided patients into two groups (AJCC Cancer Staging Manual, 7th Edition) – early stage group (stages I + II, including 19 patients) and late stage group (stages III and IV, including 23 patients). Totally, 20 of them were treated surgically while the other 22 got chemotherapy for the lack of indications of operation. Furthermore, we evaluated the chemotherapeutic effect in chemotherapy group (Response Evaluation Criteria in Solid Tumors), complete response (CR) + partial response (PR) were deemed to be effective group while stable disease (SD) + progressive disease (PD) were divided into noneffective group. Three sections of each patient were needed, one was for HE staining to confirm the diagnosis, one was for CD34 + PAS double staining, and the last one was for HIF-1α testing by using immunohistochemical method.

Immunohistochemistry

Immunohistochemical steps were in strict accordance with instructions. The main reagents including periodic acid schiff (PAS) kit, PV-6000, mouse anti-human hematopoietic progenitor cells (CD34) monoclonal antibody, rabbit anti-human HIF-1α monoclonal antibody were bought in Beijing Zhongshan Jinqiao Biological Technology Co. Ltd. (China).

CD34 + PAS double staining

After CD34 staining and diamino benzidine coloration, sections were washed with running water for 1 min, put into 5% potassium permanganate solution to oxidize for 5–8 min. Water flushed for 2 min, distilled water flushed one time. Schiff reagent stained for 10–20 min in the dark, then 0.5% sodium metabisulfite drip washed for 1 min twice, water flushed for 2 min. And then haematoxylin understained cell nucleus, alcoholic hydrochloric differentiation, back to blue, dehydration of transparent and neutral gum seal.

Evaluation of score

VM channel quantification: VM was identified by using CD34 and PAS immunohistochemical and histochemical double staining. In tumor tissue, channel that was both CD34-negative (CD34-positive appears claybank granular materials in cytoplasm or cytomembrane) and PAS staining-positive, rounded by tumor cells with or without blood cells in it was considered as a VM (Fig. 1 and 2). Necrotic and inflammatory cells were absent around the channel. The VM positive control came from normal gastric tissue specimens (Fig. 3).

MVD quantification was assessed through light microscope. Scan the whole section at low power (× 100) and find 5 areas containing most microvessels (so-called hot spots), then count the microvessels under high magnification (× 400), the average count of the 5 hot spots was considered as MVD (Fig. 2).

Immunohistochemistry estimation standard: expression was assessed by the product of staining intensity score and positive cells number percentage score. Staining intensity score: colorless means 1 point, claybank 2 points, sepia 3 points. Positive cells number percentage score: absence of positive cells represents 0 point, < 10% means 1 point, 10%–50% means 2 points, 50%–75% means 3 points, > 75% means 4 points. Immunohistochemistry-positive was affirmed if the product was more than 3, 3–6 points indicates (+), 6–9 points indicates (++), 9–12 points indicates (+++; Fig. 4).

Statistical analysis

Statistical analysis was using SPSS 17.0, methods analyzing VM with HIF-1α, clinicopathological features and stages was χ^2 test, analyzing VM with MVD was t test. $P < 0.05$ meant difference was statistically significant.

Table 1 Relationship of VM with clinicopathological features

Characters	Cases		VM		*P*
	n	%	(+)	(–)	
Sex					
Male	29	69.1	13	16	0.936
Female	13	30.9	6	7	
Age (years)					
≥ 60	23	54.8	12	11	0.320
< 60	19	45.2	7	12	
Tumor histology					
Squamous cell carcinoma	20	47.6	10	10	0.554
Adenocarcinoma	22	52.4	9	13	
T stage					
T1	3	7.1	1	2	0.589
T2	24	57.1	11	13	
T3	8	19.1	5	3	
T4	7	16.7	2	5	
N stage					
N0	21	50.0	13	8	0.030
N1–3	21	50.0	6	15	
M stage					
M0	26	61.9	15	11	0.039
M1	16	38.1	4	12	
Differentiation					
Well	1	2.4	1	0	0.223
Moderate	18	42.8	10	8	
Poor	23	54.8	8	15	

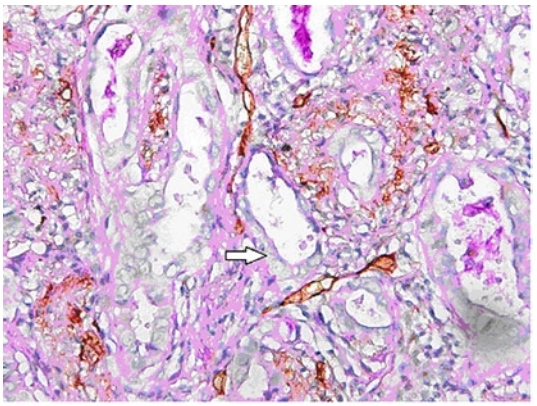

Fig. 1 White arrow showed the VM (× 400), pipeline structures with CD34-negative and PAS-positive, also red blood cells within it

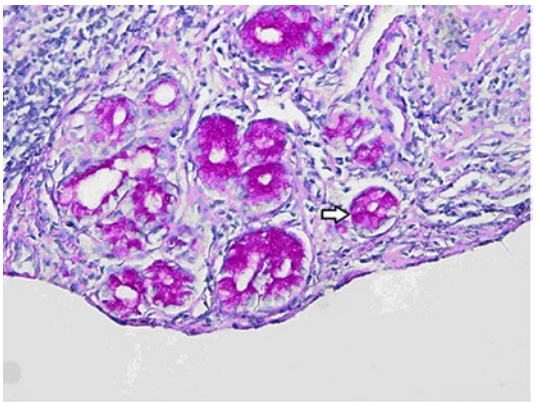

Fig. 3 PAS-positive normal gastric mucosa specimen (× 400), stomach tissue dyed red could be found (showed by the white arrow)

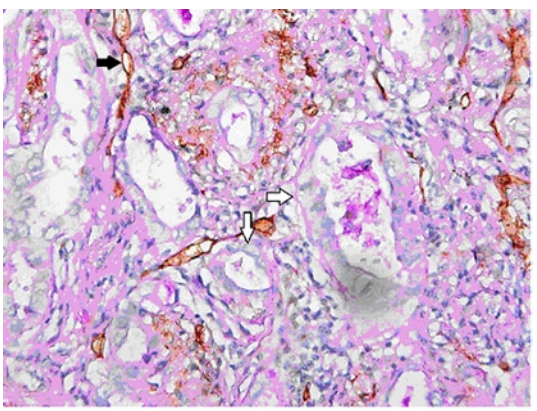

Fig. 2 White arrows showed the VM (× 400), pipeline structures with CD34-negative and PAS-positive, also red blood cells within it. The black arrow showed blood vessel with CD34-positive

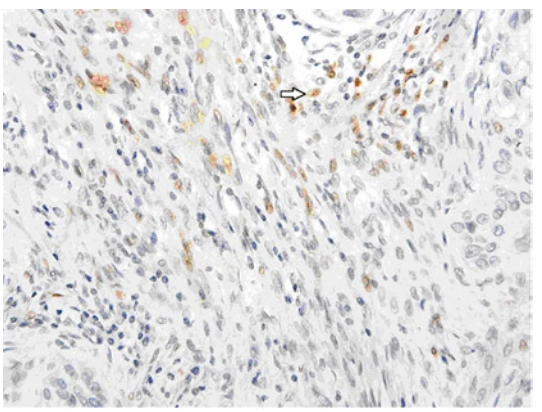

Fig. 4 HIF-1α-positive cells (× 400), cytolymph and nucleus dyed yellow were found (showed by the white arrow)

Results

Relationship between VM and stages in NSCLC

Thirteen (68.4%) VM-positive patients were identified out of the early stage group, while only 6 (26.1%) cases in late stage group were found ($P = 0.006$).

Relationship of VM and clinicopathological data in NSCLC

Details were in Table 1. Among all the factors compared, only distant metastasis and lymph nodes metastases were correlated with VM ($P < 0.05$), furthermore, distant metastasis group had a lower rate of VM-positive while lymph nodes metastases group showed the opposite result. Other clinicopathological features were found no significant difference with VM ($P < 0.05$).

VM with HIF-1α and MVD

Twelve (63.2%) cases with VM were HIF-1α positive, while only 7 (30.4%) cases without VM were HIF-1α positive ($P = 0.034$).

Comparing the VM-positive group's MVD with VM-negative group's MVD, no significative difference was found ($P = 0.846$).

VM, MVD and chemosensitivity

In late stage group, all the people got chemotherapy, 7 of them were evaluated effective, 15 patients were noneffective. Comparing VM of the two groups, 1(14.3%) case of the effective group was VM-positive, and 4 (26.7%) cases in noneffecive group were VM-positive, no significant difference was found ($P = 1.00$). Meanwhile, we found no difference in MVD between the two groups ($P = 0.827$).

Discussion

In 1971, Folkman presented angiogenesis theory, deepening our understanding of tumor, making a great difference in following therapies. Then in 1999, Maniotis [4] found another tumor microcirculation pattern,

named VM. VM is described as a kind of pipeline structure, which is formed by some tumor cells that express part of endothelial cell phenotype, allowing blood to flow through it. VM includes some characteristics: (1) no vascular endothelial cell in light microscope, electron microscope and other method consisting of immunohistochemical method; (2) PAS staining positive; (3) malignant tumor cells around; (4) red blood cells in some of VM. Studies demonstrated that VM was correlated with distant metastasis and poor prognosis, and had positive correlation with poor differentiation and late stage [10, 17, 18]. VM was also detected in lung cancer [10, 19, 20], and until now, no researches have found VM in benign tumors. So investigators considered VM as a special structure of malignancy. Meanwhile some researchers suspected VM as a retrogressive evolution of vessels when eroded by malignancy, but other studies demonstrated that VM was a functional microcirculation, without necrosis of tumor cells and inflammatory cells, communicating with vascular endothelial cells dependent vessels [21], also investigators found VM was a transition form of vessels, would be replaced by endothelial cells finally, that is three-stage theory of vessels [22]. Thus we inferred that VM was an early event of malignancy. Identically, in our study, the early stage group (stages I and II) had a bigger VM positive rate than late stage group (stages I and II), difference was significant ($P = 0.006$), and VM showed a positive correlation with lymph nodes metastasis, All were consistent with other reports [19, 23], and according with three stage theory of vessels. Meanwhile, we haven't found correlation between VM and patients' sex, age, pathological pattern, tumor size and differentiation, however, VM in distant metastasis patients was more than patients without metastasis ($P < 0.05$), that was different with other studies, reasons may be the difference in grouping. In our research, patients in stage I to stage IV were collected, including operative treatment and chemotherapy. In early stage, part of patients were diagnosed and got operation, while a lot of patients couldn't be diagnosed until losing the chance of surgery. With the progression of disease, VM was replaced by vessels, then distant metastasis happened, however, VM have already become fewer than early stage distinctly. Certainly, large sample research and follow-up study were needed. And whether patients in early stage with VM have a higher rate of distant metastases or recurrence in the future than patients without VM is still unknown, a follow-up study is needed.

Hypoxia is a common phenomenon in malignancy, investigations have demonstrated angiogenesis can be induced by hypoxia, in which Hif-1α was the main factor generated [24–26]. Sun *et al* [27] considered that Hif-1α was related to hypoxia, and the later promoted VM formation in malignant melanoma cell lines animal transplanted tumor. In our research, VM was in direct proportion to HIF-1α, meaning that VM may be a compensatory structure of hypoxia, by inducing HIF-1α and activating some pathways.

MVD is the average number of vessels in unit tissue area, considered as a quantitative indicator of angiogenesis. Investigations showed that a time window existed before the onset of anti-angiogenesis drugs when the tumor vessels tended to be normal and got a better blood supply [28–31]. Meanwhile, with the continuous of chemotherapy, effect became worse clinically, one reason may be drug fast and tumor proliferation, another one may be the injury in blood-supply-system caused by chemotherapy. Many investigations has been done to find the relationship between blood supply and chemotherapy, results were controversial. Our study in NSCLC did not show any correlation between vessels or VM and chemotherapy sensitivity. Reasons may include: (1) the difference in chemotherapy regimens (docetaxel + cis-platinum, gemcitabine + cis-platinum and pemetrexed + cis-platinum); (2) biopsy samples were too little to reflect the tumor blood supply situation; (3) samples were not enough; (4) as some researches, the chemotherapy sensitivity did not relate to tumor blood supply, or in the other hand, the benefit of increasing acreage of drugs with tumors is equal to disadvantage of drug resistance, consequently, VM and MVD cannot be a predictive factor for chemotherapy sensitivity of patients with NSCLC for now. Also no data showed any relationship between VM and endothelial cell dependent angiogenesis.

Anti-angiogenesis therapy has been investigated for long, and many targeted medicines have been in clinical application. Discovery of VM was a supplement of tumor angiogenesis, even could be a new target for the future treatment. Dose the tumor blood supply have a relationship or play a predictive role in chemotherapy sensitivity, more research is needed.

Conclusion

As a new blood supply structure of NSCLC, VM was generated during the early stage and facilitated lymph nodes metastasis. Then with the progression of disease, it may be replaced by vascular endothelial cells, so the late-stage patients especially people with distant metastases had fewer VM. But whether VM is related to the poor prognosis and recurrence in NSCLC patients is unknown, so study large sample and follow-up study is needed. As the main factor produced by hypoxia, HIF-1α may make a difference in VM formation. Thus we inferred VM might be a new target for targeted therapy, and could provide help for clinical staging and treatment.

References

1. Yoo SY, Kwon SM. Angiogenesis and its therapeutic opportunities. Mediators Inflamm, 2013, 2013: 127–170.
2. Lammers PE, Horn L. Targeting angiogenesis in advanced non-small cell lung cancer. J Nat Compr Cancer Netw, 2013, 11: 1235–1247.
3. Weidner W, Semple JP, Welch WR, *et al*. Tumor angiogenesis and metastasis correlation in invasive breast carcinoma. N Engl J Med, 1991, 324: 1–8.
4. Maniotis AJ, Folberg R, Hess A, *et al.* Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. Am J Pathol, 1999, 155: 739–752.
5. El Hallani S, Boisselier B, Peglion F, *et al.* A new alternative mechanism in glioblastoma vascularization: tubular vasculogenic mimicry. Brain, 2010, 133: 973–982.
6. Deng JH, L i HZ. Vasculogenic mimicry and mosaic vessels and targeted therapy in renal cell carcinoma. Acta Acad Med Sinicae (Chinese), 2011, 33: 462–467.
7. Ma XJ, Li J, Tan BX. Expression of HIF-1α in hepatocellular carcinoma and its relationship with vasculogenic mimicry and clinical pathology. Chinese-German J Clin Oncol (Chinese), 2013, 12: 528–531
8. Shih IeM. Trophoblastic vasculogenic mimicry in gestational choriocarcinoma. Mod Pathol, 2011, 24: 646–652.
9. Sun B, Zhang S, Zhang D, *et al.* Vasculogenic mimicry is associated with high tumor grade,invasion and metastasis, and short survival in patients with hepatocellular carcinoma. Oncol Rep, 2006, 16: 693–698.
10. Wu S, Yu L, Cheng Z, *et al.* Expression of maspin in non-small cell lung cancer and its relationship to vasculogenic mimicry. J Huazhong Univ Sci Technolog Med Sci (Chinese), 2012, 3: 346–352.
11. Wu S, Yu L, Wang D, *et al*. Aberrant expression of CD133 in non-small cell lung cancer and its relationship to vasculogenic mimicry. BMC Cancer, 2012, 12: 535.
12. Ma J, Qing Z, Su-xia H, *et al*. Role of HGK in Vasculogenic Mimicry Formation in Non-Small Cell Lung Cancer. Int J Radi Oncol, 2013, 87: S632–S633.
13. Chang Y S, di Tomaso E, McDonald D M, *et al*. Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood . Proc Natl Acad Sci, 2000, 97: 14608–14613.
14. Hendrix M JC, Seftor EA, Hess AR, *et al.* Vasculogenic mimicry and tumour-cell plasticity: lessons from melanoma. Nat Rev Cancer, 2003, 3: 411–421.
15. Sun B, Zhang D, Zhang S, *et al*. Hypoxia influences vasculogenic mimicry channel formation and tumor invasion-related protein expression in melanoma. Cancer Lett, 2007, 249: 188–197.
16. Van der Schaft DW, Hillen F, Pauwels P, *et al*. Tumor cell plasticity in Ewing sarcoma, an alternative circulatory system stimulated by hypoxia. Cancer Res, 2005, 65: 11520–11528.
17. Shirakawa K, Wakasugi H, Heike Y, *et al*. Vasculogenic mimicry and pseudo-cmedo formation in breast cancer. Int J Cancer, 2002, 99: 821–828.
18. Sun BC, Zhang SW, Zhao XL, *et al.* Pilot study of molecular mechanism on vasculogenic mimicry in Bi-directional differentiated malignant tumors. Chinese-German J Clin Oncol (Chinese), 2005, 4: 50–52.
19. Cheng ZN, Wu SW, Yu L, *et al*. The significance of vasculogenic mimicry and VEGF expression in non-small cell lung cancer. Chin J Histochem Cytochem (Chinese), 2011, 20: 343–348.
20. Wu S, Cheng Z, Yu L, *et al.* Expression of CD82/KAI1 and HIF-1α in non-small cell lung cancer and their relationship to vasculogenic mimicry. Chin J Lung Cancer (Chinese), 2011, 12: 918–925.
21. Shirakawa K, Wakasugi H, Heike Y, *et al.* Vasculogenic mimicry and pseudo‐comedo formation in breast cancer. Int J Cancer, 2002, 99: 821–828.
22. Sun Baocun, Zhang Shiwu, Zhao Xiulan, *et al*. Study on vasculogenic mimicry in malignant melanoma. Chin J Pathol (Chinese), 2003, 32: 47–51.
23. Folberg R, Hendrix MJC, Maniotis AJ. Vasculogenic mimicry and tumor angiogenesis. Am J Pathol, 2000, 156: 361–381.
24. Palazón A, Martínez-Forero I, Teijeira A, *et al.* The HIF-1α hypoxia response in tumor-infiltrating T lymphocytes induces functional CD137 (4-1BB) for immunotherapy. Cancer Discov, 2012, 2: 608–623.
25. Koh MY, Spivak-Kroizman TR, Powis G. HIF-1alpha and cancer therapy. Recent Results Cancer Res, 2010, 180: 15–34.
26. Misra RM, Bajaj MS, Kale VP. Vasculogenic mimicry of HT1080 tumour cells in vivo: critical role of HIF-1α-Neuropilin-1 axis. PloS one, 2012, 7: e50153.
27. Sun B, Zhang D, Zhang S, *et al*. Hypoxia influences vasculogenic mimicry channel formation and tumor invasion-related protein expression in melanoma. Cancer Letters, 2007, 249: 188–197.
28. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science, 2005, 307: 58–62.
29. Kerbel RS. Antiangiogenic therapy: a universal chemosensitization strategy for cancer? Science, 2006, 312: 1171–1175.
30. Zhang Q, Bindokas V, Shen J, *et al.* Time-course imaging of therapeutic functional tumor vascular normalization by antiangiogenic agents. Mol Cancer Ther, 2011, 10: 1173–1184.
31. Pries AR, Cornelissen AJ, Sloot AA, *et al*. Structural adaptation and heterogeneity of normal and tumor microvascular networks. PLoS Comput Biol, 2009: e1000394.