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Review

Sporadic and Hereditary Hemangioblastoma: The Role of Endothelial Cells

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Abstract

Hemangioblastomas (HBs) are benign, highly vascularized tumors of the central nervous system. Approximately 75% of HBs are sporadic, while 25% are associated with von Hippel–Lindau (VHL) disease. HBs consist of two main components: a rich capillary network composed of vascular endothelia and pericytes, within large vacuolated stromal cells, which harbor the genetic defect. The mechanism by which the VHL gene product (pVHL) causes HB

is not completely clear. Wild-type pVHL is involved in the response to hypoxia, targeting HIF-α for ubiquitination and degradation in the presence of oxygen, but not under hypoxic conditions. Thus, it is postulated that mutated pVHL stabilizes HIF-1α even under normoxic conditions, resulting in upregulation of cellular proliferative and angiogenic genes that promote tumorigenesis. In addition to VHL mutations, a variety of genes and microRNAs that promote angiogenesis and cell proliferation have been implicated in the pathogenesis of HB. To date, no biomarkers for the prediction of HB onset, recurrence, or progression have been identified. We recently proposed the quantification of circulating endothelial cells (CECs) and their progenitors, circulating endothelial progenitors (CEPs), as potential biomarkers for monitoring the presence of HB and its recurrence after surgical resection of the tumor. In this review, we discuss the possible role of these cells in the onset of HB and the technical challenges for their accurate identification and quantification.

1. Introduction

Hemangioblastomas (HBs) are biologically benign, highly vascularized tumors that account for 1%–2.5% of all intracranial tumors and 7%–12% of posterior fossa neoplasms in adults [1]. The majority of HBs evolve as solitary sporadic lesions. However, in 25% of cases they are associated with von Hippel–Lindau (VHL) disease (OMIM 193300), an autosomal dominant multi-organ neoplastic syndrome, with complete penetrance, and variable expression, caused by mutations in the tumor suppressor VHL gene [2, 3]. Affected patients may develop visceral tumors, such as clear cell renal carcinomas, pheochromocytomas, renal and pancreatic cysts, neuroendocrine tumors, epididymal cystadenomas, and ovarian cysts, and central nervous system (CNS) malignancies, such as cerebellar, brainstem and spinal hemangioblastomas, retinal angiomatosis, and endolymphatic sac tumors. The disease has a prevalence of 2–3 per 100 000 and an estimated incidence of between 1 in 36 000 and 1 in 52 000 live births [4, 5]. All mutation carriers usually develop clinical features by 65 years of age [6]. Although VHL disease was recognized as a specific syndrome in the early 1900s [7-9], it was not until 1993 that Latif identified the VHL gene on the short arm of chromosome 3 (3p25-26) [10]. The VHL gene encodes the VHL protein, which is mainly responsible for hypoxia-inducible factor-1 alpha (HIF-1α) degradation under normoxic conditions.

The symptoms of HBs depend on the location of the tumor. Cerebellar HBs more commonly present with headache, ataxia, nausea, vomit, dysmetria, and hydrocephalus. Brainstem HBs cause dysarthria, dysphagia, bradycardia, dyspnea, anorexia, and hiccups. Hyposthenia, hypo- or paresthesia, and pain are typical symptoms of spinal cord HBs [11]. Magnetic resonance imaging is the best diagnostic technique, through which HBs are visualized as a contrast-enhancing mass, eventually associated with a cyst or syrinx. In VHL disease, surgery is reserved to those patients who suffer symptoms, or in cases of HBs showing progressive growth in a potentially dangerous location. If the tumor is completely removed, there are usually no recurrences. However, new lesions may occur in VHL patients due to the natural history of the disease.

Although many studies have elucidated the details of the molecular pathways involved in the development of HBs in patients affected by VHL, the genetic bases of sporadic HBs are still largely unclear, and new hypotheses have also been proposed for VHL-related HBs.

2. Hemangioblastomas and Their Genetic Basis

The genetic basis of HBs has been partially clarified. VHL-related HBs are thought to be caused by the combination of a first, inherited mutation in the tumor suppressor VHL gene and a second, acquired mutation, according to Knudson's two-hit hypothesis. The VHL tumor suppressor gene is located on the short arm of chromosome 3 (3p25-26), and is expressed in several tissues both inside and outside of the CNS [12].The VHL gene coding sequence, consisting of approximately 14 500 base pairs, is formed by three exons, each comprising 712 nucleotides [13]. Two forms of VHL mRNA exist, differing in the presence or absence of exon 2, thus encoding two distinct VHL proteins, both of which function as active oncosuppressors [14]. Germ-line and sporadic mutations of the VHL gene involve all three exons, and encompass missense mutations along with non-sense mutations, micro-deletions/insertions, splice site mutations and large deletions [12]. Some studies also suggest that hypermethylation of the promoter region of VHL is a possible epigenetic mechanism of protein inactivation [15, 16]. Hypermethylation can occur in normally unmethylated sites that are rich in 5'-CG-3' sequences known as CpG islands.

The role of the VHL gene in the development of sporadic HBs has been debated, with several studies showing that VHL mutation does not cause sporadic HBs, while others indicate the involvement of the VHL gene [17, 18]. However, recent observations have confirmed that sporadic HBs may also be associated with cryptic VHL inactivation [19].

In addition to the VHL gene, other genetic alterations have also been implicated in the tumorigenesis of HBs. Comparative genomic hybridization studies showed that DNA losses at 6q are frequent alterations in HBs [20]. Other studies confirmed that loss of heterozygosity (LOH) occurs concurrently at 6q and 3p in almost 70% of cases, suggesting that a tumor suppressor gene, namely ZAC1, located at 6q is involved in the development of HB [21, 22]. The ZAC1 gene, which encodes an inducer of cell cycle arrest and apoptosis, is characterized by LOH in a significant number of sporadic HBs [20]. Moreover, in 90% of analyzed sporadic HBs cases, the ZAC1 gene promoter is hypermethylated.

3. Molecular and Cellular Features of Hemangioblastomas

As stated previously, HBs are benign CNS tumors occurring either sporadically or associated with VHL syndrome. It has been shown that mutations in the VHL gene leading to loss of its functions are involved in the pathogenesis of HBs in familial [23] as well as most sporadic cases [19].

Although genetic defects in VHL predispose individuals to HBs, the mechanisms by which the VHL gene product (pVHL) causes neoplastic transformation have not been fully elucidated. In the presence of oxygen, the wild-type pVHL degrades HIF-α by ubiquitination. In contrast, under hypoxic conditions, the wild-type pVHL does not recognize and bind to HIF-1α, thus preventing its degradation. Similarly, it is postulated that mutated pVHL stabilizes HIF-α, resulting in downstream upregulation of cellular proliferative and angiogenic genes, such as vascular endothelial growth factor and platelet-derived growth factor, which promote tumorigenesis [24]. Moreover, in

addition to VHL mutations, a variety of genes and microRNAs (miRNAs) have been implicated in the pathogenesis of HBs, all of them being functionally involved in cell proliferation and angiogenesis promoting pathways [25].

Histologically, HBs consist of two main components: a rich capillary network (composed of vascular endothelia and pericytes) within large vacuolated stromal cells, which harbor the genetic defect [12]. However, their cytological origin is uncertain and controversial. It has been hypothesized that the stromal cells are derived from embryonic, developmentally arrested hemangioblasts [26], which are multipotent common precursors of hematopoietic and endothelial cells [27]. More recently, a subpopulation of stage-specific embryonic antigen-1 positive cells has been detected. These cells are characterized by the capability of differentiating into stromal-like cells and vascular cells in the presence of the specific HB niche, and deemed to be the tumor-initiating cells in both sporadic and familial HBs [28]. Concerning vascular cells, it has been suggested that they undergo intensive reactive angiogenesis within the pseudo-hypoxic (pVHL-deficient) environment of the tumor [29]. However, *de novo* tumor-derived vasculogenesis has been reported, corroborating the hypothesis that hemangioblasts represent the neoplastic cells from which HBs originate [29].

4. Circulating Endothelial Cells and Their Progenitors

Circulating endothelial cells (CECs) and their progenitors, circulating endothelial progenitors (CEPs), are non-hematopoietic cells present in the blood. They are characterized by different origins: indeed, CECs arise from the mature endothelium of the vessel wall in response to a damaging stimulus, whereas CEPs are derived from the bone marrow and participate in vascular repair and homeostasis [30].

Phenotypically, there is a lack of consensus about the surface markers that unambiguously distinguish CECs from CEPs, mainly because they cannot be characterized by a single marker [31]. Thus, a combination of markers is required. Several markers occur on both cell types, including CD34, CD31, CD146 and CD309, while CD133 is the sole antigen that seems to be expressed on CEPs alone and is subsequently lost in mature CECs [32]. Accordingly, CECs and CEPs are usually defined by the expression of endothelial markers (such as CD34, CD31 and CD146) together with the absence of the pan-hematopoietic marker CD45 within viable, nucleated blood cells [33]; the addition of the progenitor marker CD133 allows discrimination between the two populations. Other properties that differentiate CECs from CEPs are the ability of CEPs to form colonies *in vitro* with high proliferative potential, and the uptake of acetylated low-density lipoproteins [30].

In healthy individuals, detection of CECs and CEPs in the peripheral blood is a rare event because renewal of the endothelial layer takes place continuously, but at a low replication rate of 0%–1% per day [34]. Thus, one of the major challenges to the quantification of these cells is represented by their low frequency in the blood. The recent, extraordinary advances and standardization of the technologies available for identifying, quantifying and characterizing rare populations of circulating cells [35, 36] have made the detection of CECs and CEPs possible. In particular, immunomagnetic isolation and fluorescence-activated cell sorting have been successfully employed for cell isolation, while flow cytometry has allowed multiparametric analysis [34].

Although rarely found in healthy individuals, increased levels of CECs and CEPs have been detected in patients affected by several pathological conditions, encompassing cardiovascular, autoimmune and neoplastic disorders, and correlate with disease severity [37-41]. These observations suggest that CEC and CEP numbers could be used in clinical practice as non-invasive, blood-based markers for detecting a variety of diseases and monitoring their clinical course, although the achievement of a consensus on the value of the identification and enumeration of CECs and CEPs for this purpose remains to be established.

5. Endothelial Cells in Hemangioblastomas: A Possible Predictive Role?

Although benign, HBs can be a significant source of neurological morbidity or even mortality following intratumoral hemorrhage or cystic expansion of the tumor. Surgical resection of symptomatic or progressing lesions is the preferred treatment modality and can be curative, although observation is reasonable for asymptomatic lesions with minimal growth [24]. Unfortunately, no biomarkers have yet been identified to predict the onset, recurrence, or progression of HB.

Although the studies supporting CEC and CEP enumeration and monitoring as candidate biomarkers are limited, the data obtained so far are promising. Bhatt *et al.* investigated mature CECs and CEPs, along with their ratio, in renal cell carcinoma (RCC), a condition often associated with VHL disease other than HBs [42]. A significant increase in CEPs was observed either in patients with VHL disease and RCC or in those with sporadic RCC compared to patients with VHL disease without RCC and healthy. Moreover, CEPs decreased after surgery in patients with non-metastatic sporadic RCC. Overall, these observations paved the way for the use of CEP counts as a monitoring strategy for patients with VHL syndrome [42].

In addition, more recently, we implemented a flow cytometric method for accurately quantifying CECs and CEPs in the peripheral blood of a cohort of nine patients with HB (8 VHL-affected, 1 with sporadic HB), before and after surgery, in order to assess their use as direct markers of the presence of the tumor [43]. We took advantage of a high-speed, acoustic focusing flow cytometry technique to detect staining of a panel of markers encompassing CD276, a tumor endothelial marker that characterizes a subset of CECs arising specifically from the tumor endothelium [44-46]. A higher percentage of CEPs was detected in HB patients before surgical resection of the tumor in comparison to that in healthy individuals, and the level decreased in HB patients one month after surgery, corroborating the hypothesis that baseline CEP percentage and kinetics could be a valid tool for monitoring HB onset and recurrence [43].

It is well-established that co-treatment with antiangiogenic drugs prevents the rapid mobilization of pro-angiogenic bone marrow-derived CEPs, and their subsequent tumor colonization [47, 48]. Thus, the aforementioned findings could have important clinical implications with the potential to be translated from the bench to clinical practice. However, treatment with antiangiogenic agents could importantly limit the use of CEPs as a reliable marker in surgically non-resectable HBs.

6. Concluding Remarks and Future Perspectives

Most of the studies aimed at clarifying the role of genetics in the tumorigenesis of HBs have focused on the *VHL* gene. Its biallelic inactivation is the cause of the development of the tumor in

patients affected by VHL disease. Although it is now accepted that the *VHL* gene also has a role in sporadic cases, and recent studies indicate crucial contributions of other genes in the tumorigenesis of sporadic HBs. These alterations affect several pathways downstream of the VHL protein and are involved in neovascular formation.

Moreover, recent studies showed a correlation between the presence of HBs and CEP levels, indicating a possible involvement of CEPs in the formation of HBs and a potential new predictive marker. We propose that these cells can be used as non-invasive, blood-based predictors by employing high-sensitivity flow cytometric techniques, which will overcome challenge presented by the rarity of CEPs in the peripheral blood and the need for multiparametric analysis to unambiguously identify these cells. Confirmation of the predictive role of CEPs for monitoring HBs could pave the way to the establishment of a diagnostic tool useful for neurosurgeons involved in the treatment and cure of HB.

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E.B. and A.F. conducted on line search and critical analysis of the current literature on the topic; A.D.G., L.G. and S.D.B. contributed to the writing of sections 1, 4 and 5; G.P. contributed to the writing of section 1 and 2; E.B., A.F., A.V.M., M.N., A.C. and M.P. wrote and revised the manuscript.

References

1. Brundl E, Schodel P, Ullrich OW, Brawanski A, Schebesch KM. Surgical resection of sporadic and hereditary hemangioblastoma: Our 10-year experience and a literature review. Surg Neurol Int. 2014; 5: 138.
2. Dornbos D, 3rd, Kim HJ, Butman JA, Lonser RR. Review of the neurological implications of von hippel-lindau disease. JAMA Neurol. 2018; 75: 620-627.
3. Signorelli F, Piscopo G, Giraud S, Guerriero S, Laborante A, Latronico ME, et al. Von hippel-lindau disease: When neurosurgery meets nephrology, ophthalmology and genetics. J Neurosurg Sci. 2017.
4. Maher ER, Iselius L, Yates JR, Littler M, Benjamin C, Harris R, et al. Von hippel-lindau disease: A genetic study. J Med Genet. 1991; 28: 443-447.
5. Neumann HP, Wiestler OD. Clustering of features of von hippel-lindau syndrome: Evidence for a complex genetic locus. Lancet. 1991; 337: 1052-1054.
6. Maher ER, Yates JR, Harries R, Benjamin C, Harris R, Moore AT, et al. Clinical features and natural history of von hippel-lindau disease. Q J Med. 1990; 77: 1151-1163.
7. von Hippel E. Die anatomische grundlage der von mir beschriebenen “sehr seltenen erkrankung der netzhaut”. Albrecht von Graefes Archiv für Ophthalmologie. 1911; 79: 350-377.
8. Lindau A. Studien u¨ber kleinhirncysten. APMIS. 1926; 3: 1-128.

9. Lindau A. Zur frage der angiomatosis retinae und ihrer hirnkomplikationen. Acta Ophthalmol. 1927; 4: 193-226.
10. Latif F, Tory K, Gnarra J, Yao M, Duh FM, Orcutt ML, et al. Identification of the von hippel-lindau disease tumor suppressor gene. Science. 1993; 260: 1317-1320.
11. Feletti A, Anglani M, Scarpa B, Schiavi F, Boaretto F, Zovato S, et al. Von hippel-lindau disease: An evaluation of natural history and functional disability. Neuro Oncol. 2016; 18: 1011-1020.
12. Kanno H, Kobayashi N, Nakanowatari S. Pathological and clinical features and management of central nervous system hemangioblastomas in von hippel-lindau disease. J Kidney Cancer VHL. 2014; 1: 46-55.
13. Maher ER, Kaelin WG, Jr. Von hippel-lindau disease. Medicine (Baltimore). 1997; 76: 381-391.
14. Gnarra JR, Tory K, Weng Y, Schmidt L, Wei MH, Li H, et al. Mutations of the vhl tumour suppressor gene in renal carcinoma. Nat Genet. 1994; 7: 85-90.
15. Glasker S, Bender BU, Apel TW, van Velthoven V, Mulligan LM, Zentner J, et al. Reconsideration of biallelic inactivation of the vhl tumour suppressor gene in hemangioblastomas of the central nervous system. J Neurol Neurosurg Psychiatry. 2001; 70: 644-648.
16. Meyer AJ, Hernandez A, Florl AR, Enczmann J, Gerharz CD, Schulz WA, et al. Novel mutations of the von hippel-lindau tumor-suppressor gene and rare DNA hypermethylation in renal-cell carcinoma cell lines of the clear-cell type. Int J Cancer. 2000; 87: 650-653.
17. Tse JY, Wong JH, Lo KW, Poon WS, Huang DP, Ng HK. Molecular genetic analysis of the von hippel-lindau disease tumor suppressor gene in familial and sporadic cerebellar hemangioblastomas. Am J Clin Pathol. 1997; 107: 459-466.
18. Lee JY, Dong SM, Park WS, Yoo NJ, Kim CS, Jang JJ, et al. Loss of heterozygosity and somatic mutations of the vhl tumor suppressor gene in sporadic cerebellar hemangioblastomas. Cancer Res. 1998; 58: 504-508.
19. Shankar GM, Taylor-Weiner A, Lelic N, Jones RT, Kim JC, Francis JM, et al. Sporadic hemangioblastomas are characterized by cryptic vhl inactivation. Acta Neuropathol Com. 2014; 2: 167.
20. Lemeta S, Aalto Y, Niemela M, Jaaskelainen J, Sainio M, Kere J, et al. Recurrent DNA sequence copy losses on chromosomal arm 6q in capillary hemangioblastoma. Cancer Genet Cytogenet. 2002; 133: 174-178.
21. Lemeta S, Pylkkanen L, Sainio M, Niemela M, Saarikoski S, Husgafvel-Pursiainen K, et al. Loss of heterozygosity at 6q is frequent and concurrent with 3p loss in sporadic and familial capillary hemangioblastomas. J Neuropathol Exp Neurol. 2004; 63: 1072-1079.
22. Lemeta S, Jarmalaite S, Pylkkanen L, Bohling T, Husgafvel-Pursiainen K. Preferential loss of the nonimprinted allele for the zac1 tumor suppressor gene in human capillary hemangioblastoma. J Neuropathol Exp Neurol. 2007; 66: 860-867.
23. Lonser RR, Glenn GM, Walther M, Chew EY, Libutti SK, Linehan WM, et al. Von hippel-lindau disease. Lancet. 2003; 361: 2059-2067.
24. Ordookhanian C, Kaloostian PE, Ghostine SS, Spiess PE, Etame AB. Management strategies and outcomes for vhl-related craniospinal hemangioblastomas. J Kidney Cancer VHL. 2017; 4: 37-44.

25. Mehrian-Shai R, Yalon M, Moshe I, Barshack I, Nass D, Jacob J, et al. Identification of genomic aberrations in hemangioblastoma by droplet digital pcr and snp microarray highlights novel candidate genes and pathways for pathogenesis. BMC Genomics. 2016; 17: 56.
26. Glasker S, Li J, Xia JB, Okamoto H, Zeng W, Lonser RR, et al. Hemangioblastomas share protein expression with embryonal hemangioblast progenitor cell. Cancer Res. 2006; 66: 4167-4172.
27. Choi K, Kennedy M, Kazarov A, Papadimitriou JC, Keller G. A common precursor for hematopoietic and endothelial cells. Development. 1998; 125: 725-732.
28. Ma D, Zhang M, Chen L, Tang Q, Tang X, Mao Y, et al. Hemangioblastomas might derive from neoplastic transformation of neural stem cells/progenitors in the specific niche. Carcinogenesis. 2011; 32: 102-109.
29. Zhuang Z, Frerich JM, Huntoon K, Yang C, Merrill MJ, Abdullaev Z, et al. Tumor derived vasculogenesis in von hippel-lindau disease-associated tumors. Sci Rep. 2014; 4: 4102.
30. Blann AD, Woywodt A, Bertolini F, Bull TM, Buyon JP, Clancy RM, et al. Circulating endothelial cells. Thromb Haemostasis. 2005; 93: 228-235.
31. Mancuso P, Bertolini F. Circulating endothelial cells as biomarkers in clinical oncology. Microvasc Res. 2010; 79: 224-228.
32. Bertolini F, Shaked Y, Mancuso P, Kerbel RS. The multifaceted circulating endothelial cell in cancer: Towards marker and target identification. Nat Rev Cancer. 2006; 6: 835-845.
33. Lachmann R, Lanuti P, Miscia S. Omip-011: Characterization of circulating endothelial cells (cecs) in peripheral blood. Cytometry A. 2012; 81: 549-551.
34. Erdbruegger U, Haubitz M, Woywodt A. Circulating endothelial cells: A novel marker of endothelial damage. Clinica chimica acta; Int J Clin Chem. 2006; 373: 17-26.
35. Cossarizza A, Chang HD, Radbruch A, Akdis M, Andra I, Annunziato F, et al. Guidelines for the use of flow cytometry and cell sorting in immunological studies. Eur J Immunol. 2017; 47: 1584-1797.
36. Boraldi F, Bartolomeo A, De Biasi S, Orlando S, Costa S, Cossarizza A, et al. Innovative flow cytometry allows accurate identification of rare circulating cells involved in endothelial dysfunction. PLoS One. 2016; 11: e0160153.
37. Mutin M, Canavy I, Blann A, Bory M, Sampol J, Dignat-George F. Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. Blood. 1999; 93: 2951-2958.
38. Makin AJ, Blann AD, Chung NA, Silverman SH, Lip GY. Assessment of endothelial damage in atherosclerotic vascular disease by quantification of circulating endothelial cells. Relationship with von willebrand factor and tissue factor. Eur Heart J. 2004; 25: 371-376.
39. Clancy R, Marder G, Martin V, Belmont HM, Abramson SB, Buyon J. Circulating activated endothelial cells in systemic lupus erythematosus: Further evidence for diffuse vasculopathy. Arthritis Rheum. 2001; 44: 1203-1208.
40. Mancuso P, Burlini A, Pruneri G, Goldhirsch A, Martinelli G, Bertolini F. Resting and activated endothelial cells are increased in the peripheral blood of cancer patients. Blood. 2001; 97: 3658-3661.
41. Bogos K, Renyi-Vamos F, Dobos J, Kenessey I, Tovari J, Timar J, et al. High vegfr-3-positive circulating lymphatic/vascular endothelial progenitor cell level is associated with poor prognosis in human small cell lung cancer. Clin Cancer Res. 2009; 15: 1741-1746.

42. Bhatt RS, Zurita AJ, O'Neill A, Norden-Zfoni A, Zhang L, Wu HK, et al. Increased mobilisation of circulating endothelial progenitors in von hippel-lindau disease and renal cell carcinoma. Br J Cancer. 2011; 105: 112-117.
43. De Biasi S, Gibellini L, Feletti A, Pavesi G, Bianchini E, Lo Tartaro D, et al. High speed flow cytometry allows the detection of circulating endothelial cells in hemangioblastoma patients. Methods. 2018; 134: 3-10.
44. Kraan J, van den Broek P, Verhoef C, Grunhagen DJ, Taal W, Gratama JW, et al. Endothelial cd276 (b7-h3) expression is increased in human malignancies and distinguishes between normal and tumour-derived circulating endothelial cells. Br J Cancer. 2014; 111: 149-156.
45. Seaman S, Stevens J, Yang MY, Logsdon D, Graff-Cherry C, St Croix B. Genes that distinguish physiological and pathological angiogenesis. Cancer Cell. 2007; 11: 539-554.
46. Mehran R, Nilsson M, Khajavi M, Du Z, Cascone T, Wu HK, et al. Tumor endothelial markers define novel subsets of cancer-specific circulating endothelial cells associated with antitumor efficacy. Cancer Res. 2014; 74: 2731-2741.
47. Shaked Y, Henke E, Roodhart JM, Mancuso P, Langenberg MH, Colleoni M, et al. Rapid chemotherapy-induced acute endothelial progenitor cell mobilization: Implications for antiangiogenic drugs as chemosensitizing agents. Cancer Cell. 2008; 14: 263-273.
48. Daenen LG, Shaked Y, Man S, Xu P, Voest EE, Hoffman RM, et al. Low-dose metronomic cyclophosphamide combined with vascular disrupting therapy induces potent antitumor activity in preclinical human tumor xenograft models. Mol Cancer Ther. 2009; 8: 2872-2881.