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Antiangiogenic Strategies in Medulloblastoma: Reality or Mystery

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ABSTRACT: Medulloblastoma is the most common malignant brain tumor of childhood. Surgery, radiation therapy, and chemotherapy successfully cure many patients, but survivors can suffer long-term toxicities affecting their neurocognitive and growth potential; furthermore, there is no curative therapy in up to 30% of cases, mainly because of our incomplete understanding of many of the underlying molecular and cellular processes. Angiogenesis is a hallmark of the progression of medulloblastoma and, over the last years, investigators have sought to develop effective and less toxic antiangiogenic strategies, including the inhibition or destruction of abnormal blood vessels using either antiangiogenic or vascular disrupting agents. However, the results are conflicting principally because of the complex biology of tumor vasculature and the irregular geometry of the vascular system in real space. In addition, current targets of antiangiogenic therapy, such as vascular endothelial growth factor (VEGF), are thought to be critical for both physiologic and pathologic angiogenesis, and clinical side effects of anti-VEGF therapy are beginning to emerge. We here review the state-of-the-art concerning antiangiogenic targets for medulloblastoma treatment, and discuss the complexity of the vascular system that intrinsically limits the efficacy of current strategies. **(*Pediatr Res* 63: 584–590, 2008)**

Cancer is the second leading cause of death among children between ages 1 and 14 y in the United States (1). The most common cancers in children include leukemia, brain and other nervous system cancers, soft tissue sarcomas, non-Hodgkin lymphoma, and renal tumors (1).

Medulloblastoma is an aggressive brain tumor that occurs in the cerebellum of children and young adults (2,3). The term "medulloblastoma" was originally introduced in 1910 by the American pathologist James Homer Wright (4). Bailey and Cushing (2,5) in 1925 hypothesized that medulloblastoma arises from an embryonic neuron-epithelial precursor cell, called "medulloblast." However, this cell has still not been isolated, leading Rorke (2) to include medulloblastoma in a family of primitive central nervous system (CNS) neoplasia, called neuroectodermal tumors. It has been estimated that medulloblastoma has an incidence of two to five cases per 10,000 persons for year, resulting in about 240 new cases per year in the United States (6).

Medulloblastomas predominantly arise in the roof of the fourth ventricle. They grow to invade through the ependyma in the floor of the ventricle to enter the brain stem. It has been shown that its consistent tendency is to invade the leptomeninges and to spread widely within the CNS *via* the cerebrospinal fluid (7).

Five histologic subtypes of medulloblastoma have been described (8). In the classic variant, the cells are arranged in sheets, occasionally displaying features of neuroblastic differentiation (8). Desmoplastic medulloblastomas contain nodules of tumor cells that commonly show neurocytic differentiation and are surrounded by collagen rich tissue. A third variant of medulloblastoma has been described that contains large neoplastic cells with pleiomorphic nuclei, prominent nucleoli, and abundant cytoplasm. These tumors, which are also characterized by anaplasia, are named large-cell anaplastic medulloblastomas and are associated with an especially poor prognosis (9). The melanotic and medullomyoblastoma are rare subtypes of medulloblastoma.

Despite the advances in the molecular and cellular biology of medulloblastoma, the genetic alterations involved in the majority of these neoplasia cases are poorly understood (3,10). However, it is now recognized that medulloblastoma represents a valid prototype of how deregulated developmental mechanisms can lead to tumor development and progression (11).

The most observed (in 30–50% of cases) chromosomal abnormality in medulloblastomas is iso-chromosome 17q, in which most of the short arm is lost from two chromosomes 17 and they are then fused head-to-head producing a chromosome with two centromers, little 17p and two 17q arms (12–17). A number of other chromosomal aberrations have been identified including loss of 10q (18,19).

It is indubitable that a predominant contribution to our understanding of medulloblastoma has come from the identification of two genetic syndromes exhibiting a predisposition to medulloblastoma development. Gorlin syndrome (hereditary nevoid basal cell carcinoma syndrome) and familial adenomatous polyposis syndrome arise from mutations in the human patched (PTCH) and APC genes, respectively, and both are associated with medulloblastoma formation (12). It is now well known that the gene products of these two genes

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Abbreviations: bFGF, basic fibroblast growth factor; *D*, fractal dimension; D_y, Euclidean dimension; **MVD,** micro-vessel density; **PTCH,** human patched gene; **SHH,** Sonic Hedgehog signaling pathway; **SMOH,** Smoothened gene

take part in interrelated pathways that are fundamental to neural development and cell turnover.

Other genes including Smoothened (SMOH) (20) and Suppressor of fused (SUFU) (21) have been investigated; several genes currently being explored for their potential role in medulloblastoma include the *myc* family (22,23) and the platelet-derived growth factor (PDGF) receptors and ligands (24,25).

It has been show that normal cells and cancer cells share a variable number of morphologic and behavioral properties (26). Normal embryogenesis and carcinogenesis involve changes in the proliferation, differentiation, motility and death of cells, in addition to neo-vascularization and lymphangiogenesis. Medulloblastoma originates from granule-cell progenitors that are located in the external granular layer of the cerebellum (27). This is a germinal zone harboring actively proliferating progenitor cells originating from the rhombic lip during embryonic development (10). Recent advances in understanding the molecular mechanisms involved in the control of proliferation and differentiation of these precursor cells have shed new light on the molecular pathogenesis of these cancers (10,11).

It is now widely accepted that the Sonic Hedgehog (SHH)–PTCH signaling pathway is a major mitogenic regulator of external granular layer precursor cells (10,28). In brief, Purkinje cells are the main source of the glycoprotein SHH during cerebellum development. Secreted SHH binds to the receptor PTCH, which is mainly expressed on external granular layer precursor cells and activates the pathway by relieving the inhibition of SMOH (10). This results in the activation of target genes, such as PTCH itself and the *Gli* family of transcription factors. It has been demonstrated that the crucial role of SHH in granule-cell precursor proliferation has recently been linked to cell-cycle control by the demonstration that SHH induces CyclinD1 and CyclinD2 expression during development through N-Myc (10). Progression through the G1 phase of the cell cycle in SHH-treated cerebellar granule neurons is achieved through the activation of D-Cyclins, hyperphosphorylation of Retinoblastoma protein, and activation of E2F transcription factors. It has been shown that mutations to the key mediators of the SHH–PTCH pathway (PTCH, SUFU, and SMOH) occur in 25% of sporadic human medulloblastomas (29), and germline mutations of PTCH cause a familial cancer syndrome that is characterized by medulloblastomas, basal-cell carcinomas, and rhabdomyosarcomas (10,30).

It has also been found that 14% of mice heterozygous for PTCH develop medulloblastoma, probably through haploinsufficiency of the PTCH protein (31), and murine medulloblastoma arising in mice deficient for Parp-1 and p53 (32) over-express *Gli1*, which is indicative of pathway activation. Although medulloblastoma remains a complex disease with a high mortality, these findings all suggest that probably it may arise from a deregulation of SHH–PTCH signaling in granule-cell precursors. It should be underlined, however, that up until now none of the molecular prognostic markers have been validated for routine clinical use.

CURRENT TREATMENTS OF MEDULLOBLASTOMA

It is indubitable that the recognition of molecular pathways involved in medulloblastoma development and progression could improve the clinical management of this neoplasia, a more accurate prediction of the disease risk could be achieved and new targeted treatments explored (10,11,33). It is now known that the clinical outcome of children with medulloblastoma varies according to age, postoperative tumor residuum, and metastatic stage. This has led to the development of risk-adapted treatment for this neoplasia (8). Children with medulloblastoma are currently distinguished in two groups: *a*) patients with average risk medulloblastoma which are diagnosed after the age of 3 y with nonmetastatic, and totally or near totally, excise disease, and *b*) patients who do not meet these criteria as being classified as high risk (8).

Today, surgical removal of the tumor, and adjuvant radiation therapy and chemotherapy represent the treatment of choice for patients with medulloblastoma. The standard dose of radiation therapy is 54–56 Gy to the posterior fossa and 36 Gy to the whole neuroaxis (7,34). Radiation therapy has been shown to prolong survival and result in cures. Because the cancer cells have a high potentiality to reach the cerebrospinal fluid, additional quantities of radiations are usually given to the entire brain and spinal cord. The overwhelming effects on intellectual function, academic achievement, memory, attention, and processing speed caused by current treatments especially in children younger than 8 y have been however well-documented (35,36). It has been shown that younger the child is at the time of irradiation worse is the intellectual outcome. In addition, patients whose disease recurs after combined therapy have a poor chance of being cured. It should be underlined that although different studies have shown the efficacy of high dose chemotherapy, only children with isolated local relapse, chemo sensitive disease, and minimal residual disease at the time of high-dose chemotherapy benefit.

A number of evidences suggest, however, that the prognosis of medulloblastoma is still grim in a significant proportion of patients, and novel therapeutic strategies are needed. Recently, Raffaghello et al. have investigated the expression of HLA class I antigen processing machinery component expression and function in medulloblastoma lesions, to develop effective and less toxic immunotherapeutic strategies (37). They found that multiple defects in the expression of HLA class I-related antigen processing machinery components are present in pediatric medulloblastoma, but not in pediatric noninfiltrating astrocytoma, tested as a model of well-differentiated CNS neoplasia (37). Interestingly, Ahmed et al. have demonstrated that adoptive transfer of HER2-specific T cells may represent a promising immunotherapeutic approach for medulloblastoma (38). Taken together, these findings may pave the way to future development of T cell immunotherapy of medulloblastoma using autologous tumor-specific cytotoxic T lymphocytes.

ANTIANGIOGENESIS IN MEDULLOBLASTOMA: IS IT A HELPFUL APPROACH?

Angiogenesis is a dynamic process highly regulated by a balance of pro- and antiangiogenic molecules. It is now widely accepted that the "angiogenic switch" is "off" when the effects of pro-angiogenic molecules are balanced by that of antiangiogenic molecules, and "on" when the net balance is tipped in favor of angiogenesis (39,40). Pro- and antiangiogenic molecules can be secreted from cancer cells, endothelial cells, stromal cells, blood, and the extracellular matrix (41,42), the relative contributions of which are likely to change with tumor type and site, as well as with tumor growth, regression, and relapse (39).

Several clinical trials of antiangiogenic therapies are being conducted throughout the world, but investigators are still concerned about how to achieve the maximum benefit from them and how to monitor patient response.

Although there have been advances in tumor genetics, knowledge about the tumor microvasculature in medulloblastoma is limited. As the quantification of the microvascular network is important for a better understanding of tumor angiogenesis, Gilhuis et al. performed three-dimensional reconstruction as well as morphometrical analysis of three vascular parameters (number, area, perimeter) in the main histopathological subtypes of medulloblastomas under the hypothesis that the histopathological subtype is reflected by the microvascular architecture (43).

It has been shown that the main feature of the newly generated vasculature is the structural diversity of the vessel sizes, shapes, and connecting patterns (43). Gilhuis et al. found a spectrum of patterns in the microvascular network in medulloblastoma subtypes, ranging from intensely to sparsely vascularized (43). This study clearly shows the disorganized and tortuous nature of the vasculature of some medulloblastoma subtypes (43).

Although still in the very early stages of clinical development, the combination of antiangiogenics with contemporary chemoradiotherapy regimens has emerged as a feasible and promising approach to many human cancers, including medulloblastoma. It is indubitable that targeting tumor blood vessels has become an attractive anticancer strategy. Unluckily, a large number of phase III trials using nonspecific inhibitors of angiogenesis failed to show a survival advantage (44).

Malignant brain tumors are among the most angiogenic of all human solid tumors. The principal angiogenic factors produced by medulloblastomas are vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) (45). It has been shown that VEGF expression and microvessel density (MVD) correlate directly with the degree of malignant characteristics and the overall outcome of tumors (45). One described mechanism by which VEGF and bFGF promote angiogenesis is by stimulating the activity of integrins [alpha]v[beta]3 and [alpha]v[beta]5 on endothelial cells (45–47). Integrin activation and ligand binding result in the propagation of intracellular signals, which maintain endothelial cell survival and enhance proliferation, motility, and capillary sprouting (48,49). As a consequence, targeted antagonism of [alpha]v[beta]3 and [alpha]v[beta]5 integrins inhibit brain tumorgenesis *in vivo* and may represent an important novel adjuvant therapeutic approach to brain tumors (45).

Huber et al. found that medulloblastomas produce a wide range of angiogenic factors that are, individually or together, likely to play a direct role in tumor growth (50). In 93% of the primary medulloblastoma, more than four different angiogenic factors were detected (50). In particular, VEGF165 and PDGF-A were expressed in 100%, followed by VEGF121 (89%), Ang-1 (86%), Ang-2 (79%), VEGF-C (79%), TGF-α (75%), VEGF189 (75%), and VEGF-B (57%) (50). In addition, bFGF and VEGF-C were also abundantly expressed in primary medulloblastomas (50). These findings suggest that many angiogenic peptides act in concert in the regulation of neovascularization in medulloblastoma. The ubiquitous expression of several angiogenesis stimulators in medulloblastoma indicates that antiangiogenesis therapy may provide a novel strategy that may be particularly useful for highly vascularized tumors. However, it also indicates that antiangiogenesis therapeutic strategies targeting VEGF alone may be insufficient.

Recently, a specific inhibitor of the VEGF receptor family of molecules called AZD2171 has been developed (51). Wedge et al. have shown broad-spectrum activity against a variety of adult human malignancies in preclinical xenograft models through an antiangiogenic mechanism (52). AZD2171 is being studied in a phase 1 trial by the Pediatric Brain Tumor Consortium, and other agents (*i.e.*, bevacizumab, sunitinib, and sorafenib) are in early phase clinical trials for children with cancer (51).

Application of antiangiogenic strategies to pediatric cancers presents special challenges, as the chronic administration strategies envisioned for maintaining tumor dormancy in epithelial cancers are not appropriate in growing children because of the effects on growth plates and possibly on other tissues (51,53,54).

The failure of several targeted agents, however, sent many researchers back to the bench in an attempt to clarify the discrepancy between clinical and preclinical results (43). It has been shown that angiogenesis is a hallmark of the progression of medulloblastoma and, over the last years, investigators have sought to develop effective and less toxic antiangiogenic strategies, including the inhibition or destruction of abnormal blood vessels using either antiangiogenic or vascular disrupting agents. However, the results are conflicting, principally because of the complex biology of tumor vasculature and the irregular geometry of the vascular system in real space (55–58). In addition, current targets of antiangiogenic therapy, such as VEGF, are thought to be critical for both physiologic and pathologic angiogenesis, and clinical side effects of anti-VEGF therapy are beginning to emerge (59).

Tumor vessels are structurally and functionally abnormal (39,40): unlike normal vessels, they are highly disorganized, tortuous, and dilated, and have uneven diameters and excessive branching and shunts. This may be mainly due to the heterogeneous distribution of angiogenic regulators, such as VEGF, bFGF, and angiopoietin (42,60), leading to chaotic tumor blood flow, and hypoxic, and acidic tumoral regions

(42,61–63). Moreover, although it is commonly believed that the endothelial cells making-up tumor vessels are genetically stable diploid cells (and thus different from genetically unstable neoplastic cells), tumor vasculature seems to be much more unpredictable (64).

Currently, there are no markers of the net angiogenic activity of a tumor that can help investigators to design specific antiangiogenic treatment strategies (42,65), but it is reasonable to resume that the quantification of various aspects of tumor vasculature may provide an indication of angiogenic activity (55–58).

One often-quantified element of tumor vasculature is MVD, which is used to allow a histologic assessment of tumor angiogenesis (65). The results of studies carried out over the last decade have suggested the value of using tumor MVD as a prognostic index in a wide variety of solid cancers, and it has recently been assumed that MVD may reveal the degree of angiogenic activity in a tumor (50,66–70). However, MVD has a number of substantial limitations, mainly due to the complex biology characterizing tumor vasculature (64) and the highly irregular geometry that the vascular system assumes in real space (55–58), which cannot be measured using the principles of Euclidean geometry because it is only capable of interpreting regular and smooth objects that are almost impossible to find in nature.

THE PHYSICS OF ANGIOGENESIS

Although considerable advances have been made in our molecular and cellular knowledge of the *promotion* (*i.e.*, the activation of the cell cycle, and deterioration of intercellular communications), *progression* (*i.e.*, the loss of any brake on cell division and the lack of responsiveness to external control signals), *mediation* and *inhibition* of angiogenesis, very little is known about its underlying complex dynamics. It is today recognized that vasculature and more generally tubular organs develop in a wide variety of ways involving many cell processes (71–73).

In mathematical terms, angiogenesis is a nonlinear dynamic system that is discontinuous in space and time, but advances through qualitatively different states. The word *state* defines the *configuration pattern* of the system at any given moment, and a dynamic system can be represented as a set of different states and a number of transitions from one state to another over a certain time interval (56).

At least seven critical steps have so far been identified in the sequence of angiogenic events on the basis of sprout formation: *a*) endothelial cells are activated by an angiogenic stimulus; *b*) the endothelial cells secrete proteases to degrade the basement membrane and extracellular matrix; *c*) a capillary sprout is formed as a result of directed endothelial cell migration; *d*) grows by means of cell mitoses and migration; e) forms a lumen and a new basement membrane; *f*) two sprouts come together to form a capillary loop; and *g*) second-generation capillary sprouts begin to form (56). The advancement of these different events generates a complex ramified structure that irregularly fills the surrounding environment.

The human vascular system can be geometrically represented as a fractal network of vessels that irregularly branch with a systematic reduction in their length and diameter (55–58).

Fractal objects are mainly characterized by four properties: *a*) the *irregularity* of their shape; *b*) the *self-similarity* of their structure; *c*) their noninteger or *fractal dimension*; and *d*) *scaling*, which means that the measured properties depends on the *scale* at which they are measured (55–58).

One particular feature of fractal objects is that the schemas defining them are continuously repeated at decreasing orders of magnitude, and so the form of their component parts is similar to that of the whole (58): this property is called *self-similarity*. Unlike *geometrical self-similarity*, which only concerns mathematical fractal objects in which every smaller piece is an exact duplicate of the whole, *statistical self-similarity* concerns all complex anatomical systems, including tumor vasculature. The smaller pieces constituting anatomical entities are rarely identical copies of the whole, but more frequently "similar" to it and, in such systems, the *statistical properties* of the pieces are proportional to the statistical properties of the whole (55–58).

Dimension is a numerical attribute of an object that does not depend on its process of generation, and has been defined in two ways. The first is the *topological* or *Euclidean dimension*, which assigns an integer to every point or set of points in *Euclidean space* (*E*): 0 to a *point* (defined as that which has no part); 1 to a *straight line* (defined as a length without thickness), 2 to a *plane surface* (defined as having length and thickness, but no depth); and 3 to *three-dimensional figures* (a volume defined by length, thickness, and depth). The second was introduced by the mathematicians, Felix Hausdorff and Abram S. Besicovitch, who attributed a *real number* to every natural object in *E* lying between the topological dimensions 0 and 3.

Benoit Mandelbrot uses the symbol D_γ to indicates the topological dimension, and the symbol D to indicate that of Hausdorff-Besicovitch (also called the *fractal dimension*) (55,56). The D_γ and D of all Euclidean figures are *coincident* ($D_\gamma = D$), but this is not true of fractal objects in which D is always $> D_\gamma$.

As no anatomical entity corresponds to a regular Euclidean figure, their dimension is always expressed by a noninteger number falling between two integer topological dimensions. In our case, the vascular network has a dimension lying between 2 (plane surface) and 3 (volume), and any two-dimensional section of a vascular system (as in the case of a histologic section) has a dimension lying between 0 (the dimension of a single isolated point) and 2 when the sectioned vessels entirely fill a plane surface (55–58).

Anatomical structures are also *hierarchical systems* that operate at different *spatial* and *temporal scales*, and different patterns can change, appear, or disappear depending on the scale of magnification. A fundamental characteristic is that the process operating at a given scale cannot be important at higher or lower scales.

The irregularity and self-similarity underlying *scale* changes are the main attributes of the architectural complexity

of both normal and pathologic biologic entities. In other words, the shape of a self-similar object does not change when the scale of measure changes because every part of it is similar to the original object; however, the *magnitude* and other *geometrical parameters* of an irregular object differ when inspected at increasing resolutions that reveal an increasing number of details. Experimental evidence has shown that the fractal patterns or self-similar structures of biologic tissues can only be observed within the *scaling window* of an experimentally established measure of length ϵ_1-ϵ_2, within which experimental data sets follow a straight line with a slope (1-*D*): *i.e.*, the fractal dimension remains invariant at different magnifications (56).

CONCLUDING KEY POINTS

Medulloblastoma is still today a complex disease that affects a high proportion of young people throughout the world (1,2). Although there is a general consensus that a better understanding of disease biology will allow us to develop more effective and less damaging treatments of medulloblastoma, none of the candidate molecular prognostic markers and therapeutic targets have been validated for routine clinical use (33).

This has been mainly attributed to three reasons (33):

a. The *experimental design*. Historically, molecular alterations in medulloblastoma have been identified by isolated research groups studying small number of locally and retrospectively collected tumors.
b. The *experimental technology*. Limitations in experimental technology have slowed our progress toward the identification of the molecular alterations that cause medulloblastoma. In addition, all of the identified markers have not fully validated in larger independent studies, and the relationship of these to other molecular alterations remains unknown.
c. *Clinical trials structure*. The current "three-phase" clinical trials system was designed to assess the toxicity and efficacy of combinations of cytotoxic anticancer treatments. Because the action of these agents was viewed largely as "nonspecific" then, very few resources were invested to collect tumor material for studies of tumor biology and pharmacodynamics.

Current treatment for medulloblastoma includes maximum surgical resection, whole neuroaxis radiation, and chemotherapy. Despite this aggressive treatment, only 60% of children with medulloblastoma will be cured, and most of these will suffer long-term side effects (36).

Although a large number of clinical trials of antiangiogenic therapies are being conducted throughout the world, investigators are still concerned about how to achieve the maximum benefit from them and how to monitor patient response (74).

It has been demonstrated that blood vessels play a primary role in tumor growth and metastasis, but it is now acknowledged that the structure and function of the vasculature is abnormal: the system lacks arterioles, venules, and capillaries, and the interconnections of the vessels are sometimes incomplete. Furthermore, the vessels themselves are irregularly shaped with areas of dilation and constriction, and there is no doubt that these features of the vascular system cannot be geometrically translated into Euclidean terms (55–58).

We have recently pointed out that the fractal geometry of tumor vasculature and its well-known biologic properties mean that it cannot be measured on the basis of MVD estimates alone (55–58). Our observations also support the findings of various authors who have shown the uselessness of MVD as a predictor of the efficacy of antiangiogenic treatment or as a means of stratifying patients in therapeutic trials. It has also been indicated that MVD evaluation is difficult to standardize, MVD of a biopsy does not correlate with the MVD of an entire lesion, and that changes in MVD are not necessarily induced with antiangiogenic drugs (74).

The applicability of quantitative fractal indices makes it possible to explore the range of the morphologic variability of the vasculatures that can be produced in nature, thus increasing the diagnostic importance of such variability in cancer research.

In conclusion, it can be said that:

a. Angiogenesis is a process whose large number of molecular players make it complex in time and space.
b. Analysis of the angiogenic process allows the identification of a number of different states during a certain time interval, and transitions between two successive states. However, it must be emphasized that the parameter time depends on a large number of variables that are nonlinearly interconnected in a multitude of ways, thus making it extremely difficult to predict the exact time between two states.
c. There is an increasing interest in the tumor vasculature as a potential target for antitumor therapy.
d. In medulloblastoma, clinical development of antiangiogenics alone or in combination with chemo-radiotherapy is still in its early stages.
e. A number of "molecularly targeted" antiangiogenic drugs are available, but their clinical use suffers from several relevant limitations.
f. Surrogate biomarkers of angiogenesis are needed to design preclinical studies and clinical trials involving antiangiogenic drugs, alone or in association with other therapies.
g. The spatial nonlinear advancement of the states of the promotion, progression, mediation, and inhibition of angiogenesis generates a complex ramified structure, which, in geometrical terms, irregularly fills the surrounding environment.
h. The resulting structure and function of the vasculature can be considered abnormal: it lacks an organized structure, with the absence of arterioles, venules, and capillaries; the interconnections of the vessels are sometimes incomplete; and vessels themselves are irregular in shape, with many dilated or constricted areas.
i. It is now demonstrated that the heterogeneous distribution of a large number of angiogenic regulators, such as VEGF and bFGF, lead to a chaotic tumor blood flow, and hypoxic and acidic tumoral regions.

j. The endothelial cell is a preferential target for therapy because it is common to all solid tumors. However, it should be underlined that endothelial cell phenotypes are differentially regulated in space and time, giving rise to the phenomenon of "endothelial cell heterogeneity" (59,75,76).

k. The microenvironment is critical to tumor behavior and in determining its responsiveness to such biologically directed therapies. Identifying which micro-environmental cues are responsible for coordinating these biologic outcomes is critical for our future understanding and development of antiangiogenesis strategies. Tumor angiogenesis involves the tight interplay of tumor cells, endothelial cells, phagocytes, and their secreted factors.

It has been widely shown that these conditions reduce the effectiveness of treatments, modulate the production of pro- and antiangiogenic molecules, and select a subset of more aggressive cancer cells with greater metastatic potential. There is also no doubt that these aspects and its irregular geometry make the new vasculature development and growth one of the most complex structures in biology. It is now recognized that its irregular geometry is a primary cause of the errors in visual interpretation and discordant results concerning the same tumor from different laboratories.

However, the human vascular system can be seen as a natural fractal network of vessels whose irregular branches are systematically shorter and smaller in diameter. The application of Fractal geometry to the quantification of neovascularity may therefore be more suitable to its non-Euclidean nature because irregularly tortuous contours and branching structures, such as those seen in tumoral micro-vessels, can be quantitatively characterized by their fractal dimension.

The use of fractal models of the architecture of tumor vasculature has demonstrated important implications for treatment delivery.

The careful application of fractals may have a significant impact on our understanding of the challenges of cancer treatment delivery. Quantifying the irregular structures present in tumors might help to clarify why treatment is so exasperatingly difficult, a disappointing but important finding; furthermore, we can improve our understanding of the formation of tumoral vasculature and how it differs from that of natural tissue, and thus develop more effective and directed therapies against cancer.

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