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Delivery of molecular and cellular medicine to solid tumors[☆]

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Abstract

To reach cancer cells in a tumor, a blood-borne therapeutic molecule or cell must make its way into the blood vessels of the tumor and across the vessel wall into the interstitium, and finally migrate through the interstitium. Unfortunately, tumors often develop in ways that hinder each of these steps. Our research goals are to analyze each of these steps experimentally and theoretically, and then integrate the resulting information in a unified theoretical framework. This paradigm of analysis and synthesis has allowed us to obtain a better understanding of physiological barriers in solid tumors, and to develop novel strategies to exploit and/or to overcome these barriers for improved cancer detection and treatment.

Keywords

Tumor microcirculation; Angiogenesis; Blood flow; Vascular permeability; Diffusion and convection; Receptor-ligand binding; Interstitial pressure; Lymphatics; Cell adhesion and deformation; Cancer detection and treatment

1. Introduction

Cancer is the second leading cause of death in the United States and in many industrialized countries [1]. After the primary tumor has been surgically removed and/or sterilized by radiation, the residual disease is usually managed with a variety of systemic therapies (Table 1). For these therapies to be successful, they must satisfy two requirements: (a) the relevant agent must be effective in the in vivo orthotopic microenvironment of tumors, and (b) this agent must reach the target cells in vivo in optimal quantities. The goal of our research is to examine the latter issue – the delivery of diagnostic and therapeutic agents to solid tumors and normal host tissues.

All conventional and novel therapeutic agents can be divided into three categories – molecules, particles and cells (Table 1). A blood-borne molecule or particle that enters the tumor vasculature reaches cancer cells via distribution through the vascular compartment, transport across the microvascular wall, and transport through the interstitial compartment. For a molecule of given size, charge, and configuration, each of these transport processes may involve diffusion and convection. In addition, during the journey the molecule may

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bind nonspecifically to proteins or other tissue components, bind specifically to the target(s), or be metabolized [2]. Although lymphokine-activated killer (LAK) cells (lymphocytes activated by the lymphokine interleukin-2) or tumor-infiltrating lymphocytes (TIL) are capable of deformation, adhesion, and migration, they encounter the same barriers that restrict their movement in tumors. Some of these physiological parameters are also important for heat transfer in normal and tumor tissues during hyperthermic treatment of cancer [3].

The overall aim of our research is to develop a quantitative understanding of each of the abovementioned steps involved in the delivery of various agents. More specifically, our goals are to understand: (1) how angiogenesis takes place and what determines blood flow heterogeneities in tumors; (2) how blood flow influences the metabolic microenvironment in tumors, and how microenvironment affects the biological properties of tumors (e.g., vascular permeability; cell adhesion); (3) how material moves across the microvascular wall; and (4) how it moves through the interstitial compartment and the lymphatics. In addition, we are examining the role of cell deformation and adhesion in the delivery of cells. Following analysis of these processes for molecules, particles and cells, we integrate this information in a unified framework for scale-up from mice to men (Fig. 1). In this article, I will briefly describe various experimental and theoretical approaches used in our lab, our recent findings in these six areas, and finally, how we have taken some of these concepts from bench to bedside for potential improvement in cancer detection and treatment.

2. Experimental and theoretical approaches

We have utilized five approaches to gain insight into the pathophysiology of solid tumors:

- a. A tissue-isolated tumor which is connected to the host's circulation by a single artery and a single vein [4,5]. This technique was originally developed by P.M. Gullino at the National Cancer Institute in 1961 for rats [6]; we have recently adapted it to mice [7,8] and humans [9].
- b. A modified Sandison rabbit ear chamber [10,11], a modified Algire mouse dorsal chamber [12,13], and a cranial window in mice and rats [14]. The ear chamber has the advantage of superior optical quality and the mice of working with immunodeficient and genetically engineered animals [15,16]. Recently we have developed a quantitative angiogenesis assay using these windows to study the physiology of vessels induced by individual growth factors [17] (Fig. 2). We also perfuse single vessels of tumors in these windows [18]. We also utilize two acute preparations: liver and mesentery.
- c. In vitro methods to assess the deformability, adhesion and permeability of normal and neoplastic cells [19–22], as well as measurements of adhesion molecules' expression in intact monolayers [23] (Fig. 3).
- d. Routine molecular biology techniques (e.g., in situ hybridization, Southern, Northern and Western blotting).
- e. Mathematical models to describe and integrate the data obtained from the above three approaches, to scale up biodistribution data from mice to men, and to design future experiments [24–37].

While each of these approaches has its limitations, it is their combination that has permitted us to develop the framework for tumor microcirculation and drug delivery described in this article.

3. Distribution through vascular space

The tumor vasculature consists of both vessels recruited from the pre-existing network of the host vasculature, and vessels resulting from the angiogenic response of host vessels to cancer cells [38,39]. Movement of molecules through the vasculature is governed by the vascular morphology (i.e., the number, length, diameter, and geometric arrangement of various blood vessels) and the blood flow rate [25,40–42].

Although the tumor vasculature originates from the host vasculature and the mechanisms of angiogenesis are similar [38,43], its organization may be completely different depending on the tumor type, its growth rate, and its location [42]. The fractal dimensions and minimum path lengths of tumor vasculature are different from those of the normal host vessels [40,41]. The architecture and blood flow are different not only among various tumor types but also between a spontaneous tumor and its transplants [39,44]. For example, unlike normal tissue, where RBC velocity is dependent on vessel diameter, there is no such dependence in tumors [13,14]. Further-more, the RBC velocity may be an order of magnitude lower in tumors compared to the host vessels (Fig. 4). The temporal and spatial heterogeneity in tumor blood flow may, in part, be a result of elevated geometric and viscous resistance in tumor vessels [5,45,46], coupling between high vascular permeability and elevated interstitial fluid pressure [34], and vascular remodeling by intussusception [43].

Based on perfusion rates, four regions can be recognized in a tumor: an avascular, necrotic region; a seminecrotic region; a stabilized microcirculation region; and an advancing front [47] (Fig. 5a). Intratumor blood flow distributions in spontaneous animal and human tumors are now being investigated using nuclear magnetic resonance, positron emission tomography, and functional computed tomography [30,48,49]. While limited, these results are in concert with the transplanted tumor studies: blood flow rates in necrotic and seminecrotic regions of tumors are low, while those in non-necrotic regions are variable and can be substantially higher than in surrounding (contralateral) host normal tissues [50]. Considering these spatial and temporal heterogeneities in blood supply coupled with variations in the vascular morphology at both microscopic and macroscopic levels, it is not surprising that the spatial distribution of therapeutic agents in tumors is heterogeneous and that the average uptake decreases, in general, with an increase in tumor weight. This perfusion heterogeneity also makes it difficult to heat the tumor periphery during hyperthermia [3].

4. Metabolic microenvironment

The temporal and spatial heterogeneities in blood flow are expected to lead to a compromised metabolic microenvironment in tumors. To quantify the spatial gradients of key metabolites, we have recently adapted two optical techniques: fluorescence ratio-imaging microscopy (FRIM) and phosphorescence quenching microscopy (PQM) [51–55]. As shown in Fig. 6, both pH and pO_2 decrease as one moves away from tumor vessels leading to acidic and hypoxic regions in tumors. While low pO_2 and pH are detrimental to some therapies (e.g., radiation), they might enhance the effect of certain drugs, if the drug could be delivered in adequate quantities in those regions [56–58].

To gain further insight into tumor metabolism, we have combined two powerful approaches: magnetic resonance spectroscopy and tissue isolated tumors. The former allows us to measure the energy level in tumors while the latter allows us to control the supply of individual substrates (e.g., glucose, oxygen) to the tumor. Using this approach, we have recently shown that solid tumors depend more on glucose than oxygen to maintain their ATP level [59].

5. Transport across the microvascular wall

Once a blood-borne molecule has reached an exchange vessel, its extravasation, J_s (g/s), occurs by diffusion and convection and, to some extent, presumably by transcytosis [60]. Diffusive flux is proportional to the exchange vessel's surface area, S (cm²), and the difference between the plasma and interstitial concentrations, $C_p - C_i$ (g/m). Convection is proportional to the rate of fluid leakage, J_f (m/s), from the vessel. J_f , in turn, is proportional to S and the difference between the vascular and interstitial hydrostatic pressures, $p_v - p_i$ (mmHg), minus the osmotic reflection coefficient (s) times the difference between the vascular and interstitial osmotic pressures $p_v - p_i$ (mmHg). The proportionality constant that relates transmural diffusion flux to concentration gradients $C_p - C_i$ is referred to as the vascular permeability coefficient, P (cm/s), and the constant that relates fluid leakage to pressure gradients is referred to as the hydraulic conductivity, L_p (cm/mmHg · s). The effectiveness of the transmural osmotic pressure difference in producing fluid movement across a vessel wall is characterized by s , which is close to 1 for a macromolecule and close to zero for a small molecule. Thus, the transport of a molecule across normal or tumor vessels is governed by three transport parameters (P , L_p , and S), the surface area for exchange, and the transvascular concentration and pressure gradients.

Vascular permeability and hydraulic conductivity of tumors in general is significantly higher than that of various normal tissues [14,60–65] and, hence, these vessels may lack permselectivity [66] (Fig. 7a, b). Positively charged molecules have a higher permeability [67]. Despite increased overall permeability, not all blood vessels of a tumor are leaky (Fig. 7b). Even the leaky vessels have a finite pore size, which we have been able to measure in a variety of human and rodent tumors [68], including a human colon carcinoma (LS174T) xenografted in the dorsal window (Fig. 7c, d). Our hypothesis is that the large pore size in tumors represents wide inter-endothelial junctions [69]. Not only does the vascular permeability vary from one tumor to the next, but within the same tumor it varies both spatially and temporally [60]. The local microenvironment plays an important role in controlling vascular permeability. For example, a human glioma (HGL21) is fairly leaky when grown subcutaneously in immunodeficient mice, but it exhibits blood–brain barrier properties in the cranial window (Fig. 7e, f). We have not seen such site-dependent differences for other tumors. Our working hypothesis is that the host-tumor interactions control the production and secretion of cytokines associated with permeability changes (e.g., VPF/VEGF and its inhibitors). A better understanding of the molecular mechanisms of permeability-regulation in tumors is likely to yield strategies for improved drug delivery [70].

If tumor vessels are indeed 'leaky' to fluid and macromolecules, then what leads to the poor extravasation of these agents in various regions of tumors? As shown by us and others [71–84], experimental and human tumors exhibit high interstitial fluid pressure. Furthermore, the uniformly high pressure drops precipitously to normal values in the tumor's periphery or in the peritumor region [24,31,72]. This may lower fluid extravasation in the high pressure regions, especially because the oncotic and hydrostatic pressures are also equal between the intravascular and extravascular space [73,85]. Because the transvascular transport of macromolecules in normal tissues occurs primarily by convection [60,86], convective transport of macromolecules in the center of tumors may be less than in the tumor periphery [18,24,31]. Additionally, the average vascular surface area per unit tissue weight decreases with tumor growth, hence reduced transvascular exchange would be expected in large tumors compared with small tumors [24,25].

6. Transport through interstitial space and lymphatics

Once a molecule has extravasated, its movement through the interstitial space occurs by diffusion and convection [79]. Diffusion is proportional to the concentration gradient in the interstitium, and convection is proportional to the interstitial fluid velocity, u_i (cm/s). The latter, in turn, is proportional to the pressure gradient in the interstitium. Just as the interstitial diffusion coefficient, D (cm²/s), relates the diffusive flux to the concentration gradient, the interstitial hydraulic conductivity, K (cm²/mmHg · s), relates the interstitial velocity to the pressure gradient [79]. Values of these transport coefficients are determined by the structure and composition of the interstitial compartment as well as the physicochemical properties of the solute molecule [87–93].

Using fluorescence recovery after photobleaching (FRAP) we have found D of various molecules to be about 1/3 that in water [94] and similar to that in the host tissue [88]. Similarly, the value of K for a human colon carcinoma xenograft (LS174T), measured using two different methods [95,96], was found to be higher than that of a hepatoma [93], which in turn was higher than that of the liver. Given these relatively high values of D and K , why do exogenously injected macromolecules not distribute uniformly in tumors? As discussed next, there are two reasons for this apparent paradox.

The time constant for a molecule with diffusion coefficient D to diffuse across distance L is approximately $L^2/4D$. For diffusion of IgG in tumors, this time constant is on the order of 1 h for a 100- μ m distance, days for a 1-mm distance, and months for a 1-cm distance. So, for a 1-mm tumor, diffusional transport would take days, and for a 1-cm tumor it would take months. If the central vessels have collapsed completely due to cellular proliferation and interstitial matrix rearrangement there would be no delivery of macromolecules by blood flow to this necrotic center. Binding may further retard the transport in tumors [26,27,94,97–101]. The role of binding is clearly illustrated in Fig. 8, which compares the rate of fluorescence recovery of a photobleached spot in tumor tissue injected with a non-specific vs. specific IgG. In addition to the heterogeneity in D in tumors, the most unexpected result of these photobleaching studies was the large extent (30–40%) of non-specific binding [94].

As mentioned earlier, interstitial fluid pressure is high in the center of tumors and low in the periphery and surrounding tissue [24,31,72]. Therefore, one would expect interstitial fluid motion from the tumor's periphery into the surrounding normal tissue (Fig. 5b, c). In various animal and human (xenograft) tumors studied to date, 6–14% of plasma entering the tumor has been found to leave from the tumor's periphery [60,102]. This fluid leakage leads to a radially outward interstitial fluid velocity of 0.1–0.2 μ m/s at the periphery of a 1-cm 'tissue-isolated' tumor [60]. (The radially outward velocity is likely to be an order of magnitude lower in a tumor grown in the subcutaneous tissue or muscle [24].) A macromolecule at the tumor periphery has to overcome this outward convection to diffuse into the tumor. The relative contribution of this mechanism of heterogeneous distribution of antibodies in tumors may be smaller than the contribution of heterogeneous extravasation due to elevated pressure and necrosis [24].

In most normal tissues, extravasated macromolecules are taken up by the lymphatics and brought back to the central circulation. Because of the lack of functional lymphatics within the tumor, the fluid and macromolecules oozing from the tumor surface must be picked by the peri-tumor host lymphatics [25]. To characterize the transport into and within the lymphatic capillaries, we have recently developed a mouse tail model [103]. We have measured uptake and transport in this model using a macroscopic approach (RTD analysis) and a microscopic approach (FRAP) [104,105]. Our current efforts are directed towards understanding changes in lymphatic transport in the presence of a tumor [106].

7. Transport of cells

So far we have discussed the parameters that govern the transport of molecules and particles (e.g., liposomes) in tumors. When a leukocyte enters a blood vessel, it may continue to move with flowing blood, collide with the vessel wall, adhere transiently or stably, and finally extravasate. These interactions are governed by both local hydrodynamic forces and adhesive forces. The former are determined by the vessel diameter and fluid velocity, and the latter by the expression, strength and kinetics of bond formation between adhesion molecules and by surface area of contact [107,108]. Deformability of cells affects both types of forces. Despite their importance in immunotherapy and gene therapy, the determinants of cell transport in tumors have not been examined.

Using intravital microscopy, we have recently shown that rolling of endogenous leukocytes is generally low in tumor vessels, whereas stable adhesion (> 30 s) is comparable between normal and tumor vessels (Fig. 9a, b) [109]. On the other hand, both rolling and stable adhesion are nearly zero in angiogenic vessels induced in collagen gels by bFGF or VEGF/VPF, two of the most potent angiogenic factors [17]. Whether the latter is due to a low flux of leukocytes into angiogenic vessels and/or downregulation of adhesion molecules in these immature vessels is currently under investigation. The age of the animal also plays an important role in leukocyte-endothelial interactions [110].

To gain further insight into the type of cells that adhere to tumor vessels, we examined the localization of IL-2-activated natural killer (A-NK) cells in normal and tumor tissues in mice using positron emission tomography [19,111]. Following systemic injection, we found that these cells localized primarily in the lungs immediately after injection and a non-detectable number of cells arrived in the tumor [19]. These findings were consistent with our previous work on the deformability of these cells using micropipet aspiration technique, in which we showed that IL-2 activation makes these cells rigid, and predicted their mechanical entrapment in the lung microcirculation [21,112]. Constitutive expression of certain adhesion molecules in the lung vasculature also facilitates their localization in the lungs [113].

One approach to reduce lung entrapment is to reduce the rigidity of these cells [114]. Instead, to circumvent the lung, we decided to inject A-NK cells into the blood supply of tumors, and we found that A-NK cells, both xenogenic and syngeneic, adhered to blood vessels in three different tumor models [111,115,116]. These results also supported the hypothesis that the endogenous cells that adhere to tumor vessels after systemic IL-2 injection are mostly activated lymphocytes [117].

To find out the adhesion molecules involved in the A-NK cell adhesion to tumor vessels, we utilized two in vitro approaches. In the first approach, we simulated the tumor vasculature in vitro, by incubating the human umbilical vein endothelial cells (HUVECs) in the tumor interstitial fluid collected using a micropore chamber [6,33,56,118]. Using targeted sampling fluorometry (Fig. 3), we were able to quantify the expression of relevant adhesion molecules on the HUVEC monolayers [23]. To determine the relative contributions of these molecules in adhesion under physiological flow conditions, we utilized the flow chamber [20]. Using appropriate antibodies, we found that molecules upregulated on the HUVECs include ICAM-1 and VCAM-1, which bind to CD18 and VLA-4 on the A-NK cells. We also observed sporadic upregulation of E-selectin. We were able to confirm the role of these molecules in vivo by treating A-NK cells with antibodies against CD18 and VLA-4 prior to injecting them into the arterial supply of tumors. As in our in vitro studies, blocking these adhesion molecules nearly eliminated the adhesion of A-NK cells to tumor vessels [118].

What leads to the upregulation of these molecules in the tumor vasculature? We already knew that these molecules can be upregulated by $\text{TNF}\alpha$ and a protein of 90 kDa molecular weight (p90) secreted by some neoplastic cells [107,119,120], and downregulated by $\text{TGF}\beta$ [121–123]. We wanted to find out if there are other molecules present in the tumor milieu that are also inducing this upregulation. Since tumor growth and metastasis are angiogenesis dependent, we decided to focus on the two most potent angiogenic molecules — bFGF and VEGF/VPF [38,113,124]. We found that VEGF can mimic tumor interstitial fluid, and upregulate these molecules. bFGF, on the other hand, exhibited no effect when used alone, but abrogated the upregulation induced by VEGF or $\text{TNF}\alpha$ [118]. These findings were in concert with earlier reports that bFGF retards the transmigration of lymphocytes across endothelial monolayer [125] and reduces adhesion of endothelial cells to collagen [126]. They also offer a possible explanation for lower leukocyte-endothelial interactions in tumors; bFGF might have downregulated adhesion molecules in these tumors. Our current efforts are directed towards defining interactions between angiogenic and adhesion molecules using various in vitro and in vivo approaches, including genetically engineered mice [15,113].

8. Pharmacokinetic modeling: scale up from mouse to human

So far we have analyzed each of the steps in the delivery of molecules and cells to and within solid tumors. Can we take this information and integrate it in a unified framework? We have been successful to some extent in this endeavor, using physiologically-based pharmacokinetic modeling. This approach, pioneered by two chemical engineers, K. Bischoff and R.L. Dedrick, in the 1960s, has been applied successfully to describe and scale up the biodistribution of low-molecular weight agents (for a review, see Refs. [3,127,128]). We have extended this approach to macromolecules and cells [28,29,129–131].

In this approach, a mammalian body is represented by a number of physiological compartments interconnected anatomically (Fig. 10). The volume and blood flow rate to each of these compartments/ organs are known or can be measured. The parameters that characterize transport across the sub-compartments (i.e., vascular, interstitial and cellular) and the metabolism of various agents are not generally known and cannot be easily measured. Our philosophy has been to use as many measured parameters as possible and estimate the remaining parameters by fitting the model to the murine biodistribution data. By scaling-up the parameters using well-defined scale-up laws [127], we then predict the biodistribution in human patients and compare with clinical data. Discrepancies between predictions and actual data help us in identifying inter-species differences and force us to question our model assumptions. This is an evolutionary process — as our understanding of underlying physiology and biochemistry improves, the relevant parameters are modified and the model is refined further. The model is useful not only for designing murine experiments and/or clinical trials, but also in identifying the sensitive parameters that need careful measurement and analysis. If we need detailed spatial information about a tissue/organ, then we develop a distributed parameter model for that organ, e.g., tumor [24–29,32,132,133]. While simple in principle, this cyclic approach of analysis and synthesis has served as a useful paradigm for developing a deeper understanding of drug and cell distribution in normal and malignant tissues. The level of sophistication of these models is likely to improve with our understanding of underlying principles [40].

9. Bench to bedside

The physiological factors that contribute to the poor delivery of therapeutic agents to tumors include heterogeneous blood supply, interstitial hypertension, relatively long transport distances in the interstitium, and cellular heterogeneities (Fig. 5). How can these

physiological barriers be exploited or overcome? Can we take our findings about these barriers from the bench to the bedside? Two recently developed strategies that have the potential to improve the detection and treatment of solid tumors in patients are described here.

As stated earlier, all solid tumors in patients exhibit interstitial hypertension (Table 2), provided the patient has not received any anti-edema treatment [76]. We have also shown theoretically and confirmed experimentally that IFP rises quite steeply in the tumor boundary [31,72]. We have used this knowledge in improving the design of the needle used by radiologists to localize the tumor for surgical excision [134]. We can facilitate the needle placement in a tumor by placing a pressure-sensor in the needle. Since tumors begin to exhibit interstitial hypertension almost from the onset of angiogenesis [135], this needle may be able to help in localizing early disease. The same concept may be useful in optimizing location and infusion pressure of needles employed in intratumor infusion of therapeutic agents [95], and for monitoring response to therapy [83].

Several physical (e.g., radiation, heat) and chemical (e.g., vasoactive drugs) agents may lead to an increase in tumor blood flow or vascular permeability [44,60,136–140], or lower pH [56,58]. Another approach may be based on increasing the interstitial transport rate of molecules by increasing K or D enzymatically [93,95,102] or using multi-step approaches [29,37,141,142]. We have used several physical and chemical agents to lower IFP in tumors [13,96,143–148]. Since microvascular and interstitial pressures in tumors are approximately equal, any change in one is followed rapidly by a similar change in the other, and thus the convective enhancement disappears rapidly [35,73,149,150]. By adapting a poroelastic model to solid tumors, we have calculated theoretically and confirmed experimentally that the time constant of pressure transmission across the tumor vasculature is on the order of 10 s [35]. During such a short time, the convective enhancement is calculated to be very small ($\sim 1\%$). However, if the vascular pressure is increased repeatedly and if the transvascular transport is unidirectional, or if the molecule binds avidly in the extravascular region, then we can, in principle, increase drug delivery to solid tumors significantly (Fig. 11).

In contrast, the physiological barriers discussed here may be less of a problem for: (a) radioimmunodetection; (b) treating leukemias, lymphomas, and small tumors (e.g., micrometastases) in which the physiological barriers are not yet fully established; (c) treatment of adequately perfused, low-pressure regions of large tumors; and (d) treatment with antibodies or other agents directed against the host cells (e.g., tumor endothelial cells, fibroblasts) or the sub-endothelial matrix. These physiological barriers also may pose less problems for treatment with a molecule or cell that has nearly 100% specificity for cancer cells. Until such selective molecules or cells are developed, methods are urgently needed to overcome or exploit these physiological barriers in tumors. It is hoped that an improved understanding of transport in tumors will help in developing these strategies [151].

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Fig. 1.

Quantitative understanding of various steps involved in the delivery of therapeutic agents is studied by analyzing the underlying processes and then integrating the resulting information in a unified framework. More specifically, our goal is to develop a quantitative understanding of (a) angiogenesis and blood flow; (b) metabolic microenvironment; (c) transvascular transport; (d) interstitial and lymphatic transport; (e) cell transport, and (f) systemic distribution and interspecies scale-up.

Fig. 2.

Various microcirculatory preparations used to study delivery of therapeutic agents in solid tumors: (a) Sandison window in the rabbit ear [11]; (b) Algire window in the dorsal skin of rodents [13]; (c) cranial window in rodents [14]; and (d) collagen I gel, containing angiogenic factors, sandwiched between nylon mesh (3×3 mm) to permit the growth of blood vessels [17]. These preparations allow noninvasive, continuous measurement of angiogenesis and blood flow; metabolites, such as pH, pO_2 ; transport of molecules and particles; and cell–cell interactions in vivo.

Fig. 3.

Targeted sampling fluorometry (TSF) allows the quantification of adhesion molecule expression over an intact cell monolayer on a cell-by-cell basis. At top are the two images acquired for analysis: the red nuclei are stained with propidium iodide and the adhesion molecule is labeled with fluorescein (green) using double immunostaining. The nuclei are first located in the propidium iodided channel, and regions of interest (ROIs) formed around each nucleus (bottom left); these ROIs are then applied to the immunostain image to find the fluorescence intensity in each region, corresponding to one cell. The procedure yields a histogram of intensities for the monolayer (adapted from Ref. [23]).

Fig. 4. Blood velocity as a function of vessel diameter in (a) normal pial vessels, and (b) a human glioma (U87) xenograft on the pial surface. Note that in normal microcirculation, blood velocity is dependent on vessel diameter, whereas in tumors there is no such dependence. Furthermore, the blood velocity in tumor vessels is about an order of magnitude lower than in host vessels (adapted from Ref. [14]).

Fig. 5. Physiological barriers that a blood-borne molecule encounters before it reaches a cancer cell in a solid tumor. (a) Schematic of a heterogeneously perfused tumor showing well-vascularized periphery; a seminecrotic, intermediate zone; and an avascular, necrotic central region. Note that, immediately after i.v. injection, the molecules are delivered to perfused regions only. (b) Low interstitial pressure in the periphery permits adequate extravasation of fluid and macromolecules. (c) These macromolecules move toward the center by the slow process of diffusion. In addition, interstitial fluid oozing from tumor carries macromolecules with it by convection into the normal tissue. Note that the interstitial movement may be further retarded by binding. Products of metabolism may be cleared rapidly by blood (reproduced from Ref. [102]).

Fig. 6.

Spatial gradients of metabolites in tumors. (a) pH gradients measured using fluorescence ratio imaging microscopy (adapted from Ref. [54]). (b) pO₂ gradients measured using phosphorescence quenching (adapted from Ref. [55]). Distance from the vessel wall, in microns, is shown on the x -axis, with zero being the vessel wall.

Fig. 7.

Transvascular transport in dorsal skin and tumors. (a) There is hardly any extravasation of 90-nm diameter liposomes from normal vessels; (b) heterogeneous extravasation of 90-nm diameter liposomes from LS174T tumor vessels, 48 h after injection. Note that some vessels are leaky as indicated by the yellow fluorescence for rhodamine, while others are not. Extravasated liposomes do not diffuse far from blood vessels (adapted from Ref. [14]). (c) Liposomes of 400 nm diameter (yellow fluorescent spots) extravasate adequately from LS174T tumor. (d) Liposomes of 600 nm diameter do not extravasate, suggesting that LS174T vessels have pore-size cut-off of about 500 nm (adapted from Ref. [66]). (e) The human glioma (HGL21) xenograft is permeable to Lissamin green (i.e., tumor tissue becomes green) when grown subcutaneously (Yuan and Jain, unpublished results). (f) The same glioma develops blood-brain barrier properties (i.e., impermeable to Lissamin green) when grown in the cranial window (adapted from Ref. [14]).

Fig. 8.

Role of binding in the interstitial transport in tumors, measured using fluorescence recovery after photobleaching. (a) Recovery of a photobleached spot is complete in about 100 s for a non-specific monoclonal antibody. (b) Recovery is incomplete for an antibody against carcino-embryonic antigen, present on the surface of many carcinoma cells (adapted from Ref. [94]).

(a) Rolling leukocytes at different sites

(b) Leukocyte adherence at different sites

Fig. 9.

Leukocyte-endothelial interactions in normal and tumor [109] and angiogenic [17] vessels in the dorsal skin window and the cranial window: (a) rolling, and (b) adhesion. Note that rolling is significantly reduced in tumor vessels compared to host vessels, while stable adhesion is similar in both vessel types. Both rolling and adhesion are negligible in angiogenic vessels.

Fig. 10. Schematic of physiologically-based kinetic model to describe the biodistribution of molecules and cells in a mammalian system. Such an approach permits interspecies scale-up of biodistribution (adapted from Ref. [28]).

Fig. 11.

A novel approach to increase convective transport of molecules across tumor vessels based on the finding that there is a ~ 10-s delay in the transmission of intravascular pressure to the interstitial compartment. For this approach to work, the transvascular transport has to be unidirectional or the extravasated molecule must bind avidly so that it does not intravasate when the intravascular pressure is lower than interstitial pressure (adapted from Ref. [35]).

Table 1

Systemic therapy of cancer

Therapy	<u>Agent</u>		
	Molecules	Particles	Cells
Radiotherapy	×	×	
Chemotherapy	×	×	
Immunotherapy	×	×	×
Gene therapy	×	×	×
Hyperthermia	×		
Phototherapy	×	×	

Agents used in various conventional and novel therapies can be divided in three categories: molecules, particles, and cells

Table 2

Interstitial fluid pressure (mmHg) in normal and neoplastic tissues in patients

Tissue type	<i>n</i>	Mean	Range
Normal skin	5	0.4	−1.0–3.0
Normal breast	8	0.0	−0.5–3.0
Head/neck carcinomas	27	19.0	1.5–79.0
Cervical carcinomas	26	23.0	6.0–94.0
Lung carcinomas	26	10.0	1.0–27.0
Metastatic melanomas	14	21.0	0.0–60.0
Metastatic melanomas	12	14.5	2.0–41.0
Breast carcinomas	13	29.0	5.0–53.0
Breast carcinomas	8	15.0	4.0–33.0
Brain tumors ^a	17	7.0	2.0–15.0
Brain tumors ^a	11	1.0	−0.5–8.0
Colorectal liver metastases	8	21.0	6.0–45.0
Lymphomas	7	4.5	1.0–12.5
Renal cell carcinoma	1	38.0	—

^aPatients were treated with anti-edema therapy.