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In vitro models of tumor vessels and matrix: Engineering approaches to investigate transport limitations and drug delivery in cancer☆

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ABSTRACT

Tumor–stroma interactions have emerged as critical determinants of drug efficacy. However, the underlying biological and physicochemical mechanisms by which the microenvironment regulates therapeutic response remain unclear, due in part to a lack of physiologically relevant in vitro platforms to accurately interrogate tissue-level phenomena. Tissue-engineered tumor models are beginning to address this shortcoming. By allowing selective incorporation of microenvironmental complexity, these platforms afford unique access to tumor-associated signaling and transport dynamics. This review will focus on engineering approaches to study drug delivery as a function of tumor-associated changes of the vasculature and extracellular matrix (ECM). First, we review current biological understanding of these components and discuss their impact on transport processes. Then, we evaluate existing microfluidic, tissue engineering, and materials science strategies to recapitulate vascular and ECM characteristics of tumors, and finish by outlining challenges and future directions of the field that may ultimately improve anti-cancer therapies.

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1. Introduction

Given its extensive socioeconomic impact, cancer continues to be a major focus of drug development and delivery research. Nevertheless, clinical success of anti-cancer therapies remains limited, and most treatment strategies exhibit marginal efficacy, serious side effects, and the development of resistance. Moreover, complete tumor eradication is mostly impossible, and time until patient relapse or metastasis remains a tragic measure of clinical success. Targeted therapies interfering with specific genetic and molecular mechanisms of tumorigenesis have offered improvement relative to conventional cytotoxic therapy; however, cancer cells frequently evade therapy by assuming resistance mechanisms including secondary mutations and epigenetic modifications [0–2].

While many therapies directly target tumor cells, the microenvironment in which tumor cells reside is an equally important participant in

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disease progression. During health, normal "contextual cues" of the host microenvironment prevent the cancerous outgrowth of epithelial cells [3,4]. However, perturbation of this homeostasis, e.g., due to chronic inflammation, metabolic changes, or hormonal imbalance, enables the initiation and progression of malignancy [5–8] as well as the emergence of resistance [9,10].

In addition to directly affecting tumor cell behavior, microenvironmental conditions may promote recurrence by simply preventing effective transport of therapeutics. When anti-cancer drugs are systemically administered, steps of drug delivery include transport (1) within the circulation, (2) across blood vessel walls, and (3) through the interstitial space to the tumor [11,12]. Alterations of microenvironmental conditions interfering with any of these processes may affect drug bioavailability with consequences on efficacy.

The physicochemical properties of the vasculature and the interstitial extracellular matrix (ECM) are key regulators of anti-cancer drug distribution and efficacy [13]. As the primary conduits of perfusion, blood vessels determine the availability of drugs throughout the body and within individual tissues. However, heterogeneous microvascular function as present within tumors can compromise delivery and undermine the effects of therapeutic agents [13]. Enhanced permeability and retention (EPR) in leaky vessels has facilitated the targeting of macromolecular therapies [14–18]. Yet, the asymmetric distributions of oxygen or drugs within a tumor provide a conducive landscape for the evolution of resistance within heterogeneous populations of cancer cells [19]. Although vascular structure and function largely regulate the spatiotemporal distribution of drug, interstitial space can also affect transport rates [20]. In particular, excessive ECM deposition due to fibrotic remodeling (also termed desmoplasia) physically hinders diffusion of large anti-tumor molecules through the interstitium [20].

Despite the well-established physical principles governing biological transport phenomena, the opportunity to leverage these principles to improve therapeutic outcomes is limited. Conventionally, new anti-cancer compounds are first tested in 2D tissue culture, which provides homogeneous access to drug and neglects the 3D microenvironmental properties inherent to tumors. Additionally, even positive results from animal studies do not always translate to efficacy in humans due to species-dependent discrepancies in signaling and physiology [21,22]. The development of tissue-engineered model systems that accurately recapitulate human tumor with increasing physiological complexity may help to understand and test microenvironmental parameters affecting tumor response. Here, we review current understanding of the biological characteristics underlying tumor-associated changes of the vasculature and ECM properties, examine the consequences of these parameters for mass transport and drug delivery, and present emerging in vitro strategies that may provide new insights for improved anti-cancer therapies.

2. Tumor vasculature: biophysical changes and their relevance to drug delivery

2.1. Biological characteristics of tumor microvasculature

Since Judah Folkman's seminal observations in 1971 that tumorigenesis is associated with the ingrowth of abnormal blood vessels [26,27], vascular dysfunction has become an enduring theme in cancer biology and anti-cancer therapy. When compared to healthy vasculature, tumor vessels are leaky, fenestrated, tortuous, and dilated, with chaotic branching patterns including shunts and loops, as well as irregular hierarchy of vessels [28–33] (Fig. 1). The tumor vasculature comprises at least six types of vascular structures with distinct properties, including mother vessels (MVs) and several varieties of daughter vessels (capillaries, glomeruloid microvascular proliferations, vascular malformations) [34,35]. MVs are enlarged sinusoids resulting from pericyte detachment, basal membrane (BM) degradation, and endothelial thinning [35]. Despite this distension, MVs do not increase blood flow, possibly due to their hyper-permeable membrane function [34]. Meanwhile, immature daughter vessels are fenestrated and lack functional perfusion. Vascular leakage, coupled to dysfunctional lymphatic drainage, results in an accumulation of interstitial fluid, which compromises the hydrostatic and osmotic pressure gradients that drive transvascular convection [36]. Collectively, this heterogeneous network of aberrant blood vessels yields erratic perfusion of diseased tissue, with important consequences for pathogenesis and therapy.

Hypoxia and acidosis are the most prominent consequences of deregulated vascular function. Compromised vessel characteristics lead to poor supply of oxygen and clearance of metabolic wastes, mediating sustained hypoxia (<1% O_2) and acidosis (as low as pH ~6.6–6.8 in some areas) [37–39]. These microenvironmental conditions directly promote tumor progression and new vessel formation by activating a variety of transcriptional programs [40–43]. In particular, stabilization of the alpha subunit of the hypoxia inducible factor 1 (HIF-1) transcription factor in low-oxygen conditions leads to an orchestrated program of hypoxic response [41,44–46] that includes the up-regulation of pro-angiogenic morphogens (including vascular endothelial growth factor [VEGF] and basic fibroblast growth factor [bFGF]). However, rather than promoting the formation of healthy blood vessels that could normalize tissue O_2 levels and pH, excess pro-angiogenic signaling activates a vicious cycle that undermines vascular stability by impairing vessel organization and permeability thus exacerbating these pathological conditions [47–49].

In addition to metabolic transport, impaired vascular function also compromises the homogeneous delivery of therapeutic agents, resulting in poor distribution of drugs throughout the tumor [50]. Low vascular function at the tumor interior prevents therapeutic access to large regions of tissue [30]. Homogeneous delivery is further undermined by the absence of hydrostatic and osmotic pressure gradients, which are necessary for interstitial convection to distribute large therapeutic agents [11,52]. In some cases, enhanced vascular permeability and retention (EPR) has been coopted as a mechanism for tumor targeting of large particles such as antibodies and micelles [14]. However, although high molecular weight drugs and drug carriers easily traverse the endothelial membrane of the tumor, they have poor penetration depth and achieve little benefit in regions distal to blood vessels [50,53,54]. Therefore, vascular normalization is an emerging theme for improving therapeutic delivery [55–57].

Whereas most vascular therapies emphasize the destruction of blood vessels to stunt tumor growth, some researchers hope to re-appropriate angiogenic drugs as adjuvant therapy in order to improve drug distribution [58]. By increasing perfusion in the tumor, this method may overcome major disadvantages of chemotherapy such as short half-life and small therapeutic index (range of concentration between efficacy and toxicity) [59]. In the past decade, numerous clinical trials have revealed the benefits of anti-angiogenic therapy as an adjuvant to chemotherapy or radiation therapy [59–61]. However, the dearth of pre-clinical models to recapitulate vascular transport remains a challenge for the development of new strategies for vascular normalization.

2.2. Effects of microvascular dysfunction on transport physics

Whereas conventional pharmacokinetic metrics (e.g. distribution volume, half-life, clearance rate) characterize the average availability of drug in tissues, these measurements only poorly approximate the effective distribution of agents within tumors. In fact, large regions of tumors lack adequate vascular perfusion and thus, can remain unaffected by treatment, even at high doses. This is particularly true of large molecules (>10 kDa), which are not able to freely diffuse across the endothelium and through the tissue. Instead, convective forces govern the exchange of large compounds from the circulation (Box 1). In healthy tissues, these forces balance the influx and outflux at the arterioles and post-capillary venules to provide adequate perfusion. In the case of a tumor, however, vascular leakiness reduces hydrostatic pressure in the vessel while increasing osmotic pressure in the