Advanced Drug Delivery Reviews 97 (2016) 270–279

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

In vitro cancer cell–ECM interactions inform *in vivo* cancer treatment☆

Andrew W. Holle [a], Jennifer L. Young [a], Joachim P. Spatz [a,b,*]

[a] Department of New Materials and Biosystems, Max Planck Institute for Intelligent Systems, Stuttgart 70569, Germany
[b] Department of Biophysical Chemistry, University of Heidelberg, Heidelberg 69047, Germany

ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 5 October 2015
Accepted 11 October 2015
Available online 17 October 2015

Keywords:
Extracellular matrix (ECM)
Chemotherapy
Chemoresistance
Metastasis
Tumor microenvironment
Ligands

ABSTRACT

The general progression of cancer drug development involves *in vitro* testing followed by safety and efficacy evaluation in clinical trials. Due to the expense of bringing candidate drugs to trials, *in vitro* models of cancer cells and tumor biology are required to screen drugs. There are many examples of drugs exhibiting cytotoxic behavior in cancer cells *in vitro* but losing efficacy *in vivo*, and in many cases, this is the result of poorly understood chemoresistant effects conferred by the cancer microenvironment. To address this, improved methods for culturing cancer cells in biomimetic scaffolds have been developed; along the way, a great deal about the nature of cancer cell-extracellular matrix (ECM) interactions has been discovered. These discoveries will continue to be leveraged both in the development of novel drugs targeting these interactions and in the fabrication of biomimetic substrates for efficient cancer drug screening *in vitro*.

Contents

1. Introduction

The diverse nature of cancer evinces the great variety of relevant biochemical pathways—the loss of differentiation in cancer cells in many ways shares functional characteristics with stem cells and development. A number of strategies have been pursued to both prevent and treat cancer, each relying on a different facet of the disease. Of the

☆ This review is part of the Advanced Drug Delivery Reviews theme issue on "Extracellular Matrix (ECM) and ECM-like materials: Therapeutic Tools and Targets in Cancer Treatment".

* Corresponding author at: Department of New Materials and Biosystems, Max Planck Institute for Intelligent Systems, Stuttgart 70569, Germany.
E-mail addresses: holle@is.mpg.de (A.W. Holle), young@is.mpg.de (J.L. Young), spatz@is.mpg.de (J.P. Spatz).

http://dx.doi.org/10.1016/j.addr.2015.10.007

approximately 175 individual cancer drugs currently approved by the FDA, the majority are targeted toward DNA replication and repair pathways (e.g. alkylating agents, antimetabolites, anthracyclines, topoisomerase inhibitors, or mitotic inhibitors [1]). A number of recently approved drugs are angiogenesis inhibitors [2], but to date, a drug targeting a cancer cell's interactions with the extracellular environment has not been approved, despite the fact that cancer's relationship with the extracellular matrix (ECM) has received increasing scrutiny in the lab. Specifically, the ways in which cancer cells interact with the physical properties of the ECM, how cancer cell–ECM attachment can directly affect treatment efficacy, and the development of modern drug strategies taking advantage of the cancer–ECM relationship are all gaining importance. The goal of this review is to provide a comprehensive overview of A) important breakthroughs in the understanding of physical cancer–ECM interactions *in vitro*, B) the increasing evidence that these interactions can abrogate the effects of chemotherapeutic intervention, and C) the current state of cancer-ECM-based drug discovery and development, with a special focus on how basic science discoveries have informed this pipeline.

2. The physical relationship between cancer cells and the extracellular matrix *in vitro*

The extracellular matrix (ECM) is a highly complex fibrous mesh composed of proteins (e.g. collagen, laminin, fibronectin, elastin), glycosaminoglycans (e.g. hyaluronic acid, heparin), proteoglycans (e.g. perlecan, syndecan), and sequestered growth factors. The ECM not only plays an important role in providing support to tissues but also directs a diverse set of functions in individual cells. Physical properties of the extracellular matrix like stiffness, topography, and ligand presentation have been shown to affect a variety of cellular responses [3–5]. Adhesion of cells via specific receptors, mainly integrins, to the ECM stimulates signaling pathways that regulate survival, proliferation, migration, polarity, and differentiation [6–8]. Each of these properties of cancer cell–ECM interaction can and have been modeled by researchers *in vitro* (Fig. 1) in the pursuit of a more faithful mimic of the *in vivo* tumor microenvironment [9–12] As both chemical and physical characteristics of the ECM influence cell behavior, and these specific properties can be altered in cancer, a better understanding of cancer ECM composition and subsequent cellular responses are required [13–15]. The well-characterized action of matrix metalloproteinases (MMPs) bear mentioning despite being beyond the scope of this review; examinations of both *in vitro* basic research and MMP-centered clinical trials can be found in several excellent reviews [16–18].

2.1. Stiffness

Matrix rigidity has been shown to influence cellular migration [19–22], adhesion [23–25], proliferation [26–28], and differentiation [29,30]. Cellular sensing of rigidity is a complex process integrating multiple mechanosensitive pathways [31]; thus, the use of substrates in which rigidity can be precisely tuned to mimic that of both healthy and cancerous tissue is important in presenting physiologically relevant properties to cells *in vitro* [32].

In the context of cancer biology, ECM stiffness and cellular stiffness, i.e. the elastic moduli of individual cells, are intertwined, as the difference between these two material properties can define the nature of their interaction. Metastatic cancer cells have consistently been shown to be softer than benign cells, with some cell types exhibiting stiffnesses an order of magnitude lower than their healthy counterparts [33–36]. This could be the result of impaired keratin organization observed in a number of cancer cell types [37], as keratin reorganization prompts a sharp drop in individual cell stiffness [38]. This is in contrast to tumor-associated ECM, which has been found to be generally stiffer than surrounding healthy tissue [39–42]. There are a number of contributing factors potentially at play in malignant matrix stiffening, including fibrosis [43,44] and the increase in interstitial pressure due to tumor growth and chaotic microvasculature [45]. Additionally, collagen networks have been shown to stiffen in breast cancer (BC) [46], which is likely due to the crosslinking role of the enzyme lysyl oxidase (LOX). LOX has been found to be upregulated in breast tumors and may play a role in focal adhesion kinase (FAK) activation and cell-matrix adhesion [47]. Increases in collagen network deposition and/or stiffness have been shown to stimulate integrin signaling and cancer cell proliferation [44,48], further reinforcing the role of substrate mechanics in cancer progression.

There are a number of ways in which researchers can study the effects of substrate stiffness on cancer cells *in vitro*. The use of ECM-based natural biomaterials like collagen gels, hyaluronic acid (HA), and Matrigel allows for a degree of biomimicry, and each has its own protocol for controlling stiffness. For instance, collagen gels can be attached to a stiff substrate or used free-floating [48], combined with

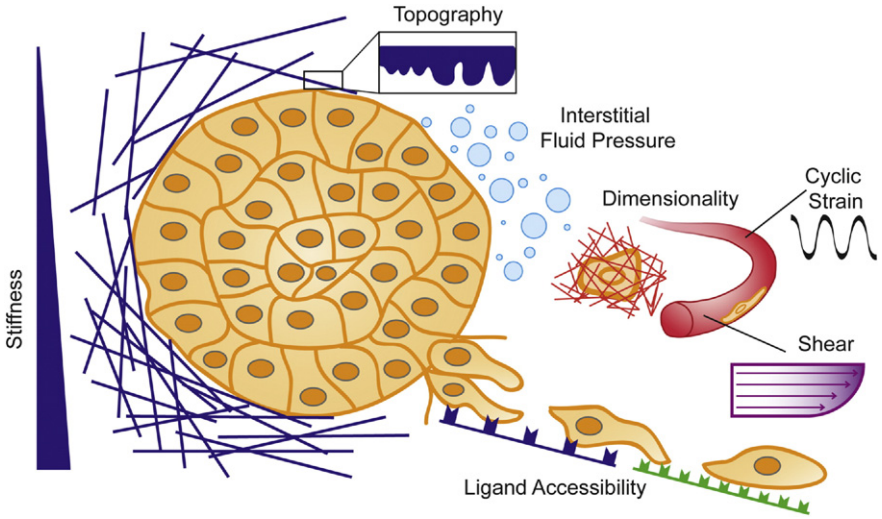

Fig. 1. *Tumor/cancer cell–ECM interactions in vivo that can be modeled in vitro.* In the tumor microenvironment, a number of physical characteristics of the ECM can influence cancer cell or tumor behavior. In tumor microenvironments, the ECM tends to be stiffer, usually as a result of increased collagen crosslinking. Deposition of ECM fibers like collagen contributes to the topography of the ECM, as well as dictates which ligands will be accessible to the cells. Interstitial pressure also rises due to poorly functioning lymphatics and chaotic microvasculature caused by enhanced angiogenesis. Cells can be exposed to three-dimensional ECM in the stroma and two-dimensional ECM upon intravasation into the circulatory system, where they are also subject to shear forces and cyclic strain.

different levels of agarose [49], or simply fabricated with more or less collagen fibers [50] to control the level of elasticity felt by the cells.

Synthetic hydrogels composed of polyacrylamide (PA), polyethylene glycol (PEG), or polydimethylsiloxane (PDMS), while not as biomimetic as natural materials, allow for more precise control over substrate stiffness. The stiffness of PA gels can be varied by altering the ratio of crosslinker to monomer [19,51]. PEG gels also take advantage of crosslinker to monomer ratios and/or photoinitiator systems to control stiffness and have been used in 2D or 3D formats to analyze stiffness-dependent cancer cell drug responses and migration patterns [52,53]. PDMS stiffness can be tuned by altering the ratio of base to curing agent and has also been used to analyze cancer cell behavior in response to different elasticities [54]. One current limitation of *in vitro* models for substrate stiffness is the fact that stiffness *in vivo* can be heterogeneous over nano- and micro-scales [55], likely due to heterogeneous matrix deposition at focal regions of tissue.

2.2. Ligand accessibility

Cell adhesion and behavior have been shown to be sensitive to ligand presentation at the nano-scale (i.e. chemistry, density, geometry) [56–60]. Specific ligands presented to cells in the tumor microenvironment depend on the protein composition of the ECM. This composition has been shown to control cell proliferation, migration, adhesion, and viability [61], as well as the expression of pro-survival pathways, allowing cells to escape drug-based treatment [62]. Tumor development is often associated with the upregulation of different ECM proteins; collagen VI expression, which correlates with tumor grade in ovarian cancer patients, has been shown to be upregulated in drug-resistant cancer cells, which can subsequently remodel the ECM to further promote chemoresistance [63].

By controlling the ECM composition *in vitro*, important observations on the nature of ligand interactions can be made. Nanostructured, flexible materials are useful for examining adhesive properties and resultant cellular responses in cancer cells and can be synthesized via a block copolymer micelle nanolithography (BCML) technique, followed by transfer nanolithography onto soft substrates, which have been extensively described in a variety of systems [64–67]. This system allows for the direct control of ligand composition and spacing, and ongoing research is focusing on the relationship between ECM ligand motifs and chemoresistance. Directly coating glass surfaces with biotin allows for direct conjugation of ECM ligands; this system has been employed to better understand the relationship between RGD (arginylglycylaspartic acid) and EGF (epidermal growth factor) ligands. By controlling the proportion of the two motifs, it has been shown that EGF directly affects cancer cell focal adhesions, suggesting a connection between integrin attachment and EGF receptor signaling [68].

Ligands can also be presented in a less controlled manner by adjusting the proportion of different ECM proteins available for cancer cells to interact with. When cells are plated in three-dimensional matrices composed of the ECM protein laminin, an increase in DNA methylation is observed, resulting in a more invasive cancer cell phenotype [69]. Hyaluronic acid hydrogels supplemented with fibronectin, laminin, or cyclic-RGD have been used to illustrate this effect, as laminin-rich gels conveyed resistance to ERK (extracellular-signal-regulated kinase) inhibition and fibronectin-rich gels conveyed resistance to BRAF (proto-oncogene B-rapidly accelerated fibrosarcoma) inhibition [62]. The availability of collagen or fibronectin in the *in vitro* microenvironment has also been shown to alter cancer cell stiffness, suggesting a level of crosstalk between ligand accessibility and cell mechanics [70]. Other systems have been built that allow for combinatorial analysis of variable ECM composition and thus ligand composition [71], showing the first steps towards a truly high throughput biomimetic drug screening platform.

2.3. Dimensionality

While the majority of *in vitro* cancer cell experimentation has been performed on two-dimensional substrates, recent advances in biomaterials have allowed for increasing investigation of the behavior of cancer cells and tumors in three-dimensional scaffolds. Cancer cells can be distinguished from normal cells in three-dimensional culture by their growth rates and morphologies; while normal cells reduce their rate of proliferation and maintain their spatial position, malignant cells generally will grow rapidly and invade into surrounding regions [72], to the point where morphology in some 3D scaffolds can predict malignant potential [73]. Even among cancer cells, a morphological and behavioral gradient exists, with more highly malignant cell lines exhibiting stellate morphology and invasive processes compared to grape-like morphology in less malignant lines [74]. Clusters of cancer cells known as multicellular tumor spheroids (MCTs) cultured in these matrices can be used as *in vitro* models for early tumor architecture, tumorigenic gene expression, and drug response, with invasion out of MCTs into the surrounding 3D scaffold mirroring early metastatic events [75]. Genes expressed in these reproductions of early tumor structure have been correlated with genes upregulated by cancer cells *in vivo*, validating the mimetic nature of such models and shedding new light on difficult-to-observe events during tumorigenesis *in vivo* [76].

In order to study the nature of 3D ECM-cancer cell interactions, sufficiently biomimetic substrates must be fabricated. A number of strategies have been pursued, including the use of the 3D scaffold Matrigel [75], a basement membrane preparation extracted from mouse sarcoma cells *in vitro*. 3D scaffolds composed of collagen type I have been used to present stiffness gradients to cancer cells *in vitro*, allowing for the observation that cancer cells infiltrate more extensively into soft 300 Pa regions than into stiffer 1.2 or 6.0 kPa regions [77]. Distinctly different phenotypes of cancer cell invasion have been observed on 2D and 3D substrates, with cells in unconfined lines exhibiting 'push-and-pull' behavior characteristic of mesenchymal invasion, and cells in confined microchannels moving in an amoeboid-like sliding fashion [78]. Three-dimensional silk sponges have also been used to analyze cancer cell behavior *in vitro*, with significant differences observed in the expression of angiogenic factors compared to two-dimensional substrates [79]. Controlled comparisons between 2D and 3D substrates have also been performed with fibronectin matrices, allowing for the observation that integrin binding and MT1-MMP activity are required in two dimensions, but not in three, illustrating the purported switch from mesenchymal to amoeboid cancer invasion [80]. Pseudo-3D substrates sandwiching cancer cells in between two 2D substrates allow for precise control over ligand presentation, making detailed analysis of focal adhesion complexes and migration speed possible [81]. While the field has recognized that 3D scaffolds generally present a more biomimetic environment necessary for modeling cancer cell–ECM interactions and screening cancer cell–drug outcomes, 2D substrates have not lost all of their usefulness. *In vivo*, several cancer-relevant microenvironments are better modeled by 2D substrates, most notably the boundary between ECM and the circulatory system, but also including any 2D interface in which a cell will preferentially bind to a flat surface.

2.4. Topography

Surface topography refers to the nanometer or micrometer-scale orientation of ECM components, including fibrils and other supramolecular structural patterns [82,83]. These patterns have been shown to guide cancer cell migration in the tumor microenvironment, as analysis of breast cancer explants shows that tumor cells and cancer-associated stromal cells migrate along reassembled collagen fiber 'highways' exhibiting macroscopic topography [84]. Changes in the native mesh-like topography of collagen ECM into radially aligned fibers have been observed *in vivo* [85], and *in vitro* experimentation has confirmed that outwardly radiating collagen fiber patterns can promote invasive

behavior while randomly aligned fibers reduces it [86]. It has been speculated that ECM topography is linked to intracellular signaling in cancer cells, likely through the physical arrangement of key integrin receptors and focal adhesions [87]. ECM porosity, often represented as the average cross-sectional diameter of the gaps in the fibers comprising the ECM, is another form of topography that plays a role in cancer migration and invasion [88]. When matrix pore size is smaller than the width of the cell, ECM degradation is triggered in order to alter the matrix topography, thereby allowing invasion and migration [89]. Like other forms of topography, porosity is a direct result of patterns in ECM protein deposition, and thus has a great deal of overlap with studies examining ligand accessibility as a function of protein deposition [50,90].

2.5. 'Outside-in' forces: Interstitial fluid pressure, cyclic strain and shear stress

The nature of forces that act upon cancer cells, or 'outside-in' forces, is also altered compared to healthy tissue. Interstitial fluid pressure (IFP) in the tumor microenvironment, which is predictive of patient outcome [91] (although conflicting results addressing whether increased IFP is associated with increased or decreased long-term survival have been reported, perhaps due to tissue type [91,92]), can be at least an order of magnitude higher than normal tissue due to abnormal microvasculature and poorly functioning lymphatics [45]. Individual cancer cells have been exposed to different levels of hydrostatic pressure in the lab, leading to the discovery that increased interstitial fluid pressure *in vitro* is sufficient to alter angiogenic gene expression, a key regulator of the chaotic microvasculature responsible for aberrant IFP *in vivo* [93,94], with certain cell lines displaying an increased sensitivity to chemotherapeutic drugs while exposed to pathological IFP [92], suggesting that the physical barrier to drug penetration is the driving factor in IFP-induced chemoresistance [95]. Collagen gels under volumetric compaction have also been used to mimic increased IFP [96] and monitor subsequent changes in signaling pathways.

Shear stress, imposed onto cancer cells during intravasation into the circulatory system after escaping the tumor microenvironment, is another example of a physiological force acting on cancer cells [97]. During this process, invasive cancer cells must adhere to vessel walls; otherwise, they will continue to recirculate and be susceptible to death by shear stress [98]. This process must be more closely examined to better understand why so many intravasating cells fail to extravasate, a phenomenon thought to contribute to the aggressive nature of cancer cells that survive the circulatory system [99]. To mimic these conditions *in vitro*, cancer cells can be plated onto flat substrates and spun at 60 rotations per minute [98]. Microfluidic devices and Darcy flow chambers have been used extensively to provide both shear stress and chemoattractant gradients, revealing different patterns of cancer cell migration in response to different magnitudes of shear force [100,101]. Cone and plate viscometers have also been used to demonstrate that shear stress can affect the sensitivity of circulating cancer cells to drug treatment [102].

The cyclic forces present in the circulatory system, which have historically been an important focus of research due to the strong role of angiogenesis in tumor growth, have also been shown to contribute to mechanosensitive processes in cancer cells. Tumor-derived endothelial cells have been examined under 10% uniaxial cyclic strain and found to exhibit aberrant Rho-mediated mechanosensitivity, likely due to reprogramming caused by exposure to the tumor microenvironment [103].

Taken together, it is clear that there are a wide variety of interactions and forces that cancer cells experience *in vivo*. Recently, an increasing number of groups are taking advantage of this knowledge and applying *in vivo* principles to the engineering design of *in vitro* scaffolds and systems. Ultimately, near-perfect reconstruction of these *in vivo* conditions will allow researchers to more accurately conceive, design, and test strategies for treating cancer. However, due to the current use of less-than-ideal materials, we are learning more about the unforeseen outcomes of chemotherapeutic treatment *in vivo*.

3. Evidence for the ECM altering drug treatment

There are multiple ways in which a cancer cell or tumor can obtain resistance to drug treatment, including gene mutations, gene amplification, or epigenetic alterations that impact how cells interact with various drug compounds [104]. Several types of cancer cells have been shown to utilize their interactions with the surrounding environment to acquire drug resistance, either within the primary tumor (e.g. multiple myeloma cells within the bone marrow), or within the context of disseminating metastases to distant organs (e.g. metastasis of breast or prostate cancer cells to bone) [105–110]. These interactions can be loosely grouped into physical barriers to treatment (hypoxia, pH, and interstitial fluid pressure) and cell-adhesion-based drug resistance (ECM organization and protective ligand binding) (Fig. 2).

3.1. Physical barriers to treatment

In many cases, drug delivery in the interstitial spaces in and around a tumor relies on diffusion and pressure-driven convection [111]. As a result of this, cancer cell-driven remodeling of the ECM provides the simplest resistance to treatment in the form of physical barriers that delay or abrogate drug delivery [112]; indeed, most anticancer drugs show limited penetration into solid tumors [104,113]. A number of strategies have been proposed to mitigate the barrier-like effects of the ECM in cancer drug delivery [95].

The tumor microenvironment is extremely heterogeneous, with gradients in the degree of hypoxia, pH, and ECM composition, all of which can influence the efficacy of drug treatment for several independent reasons [104]. Hypoxia results from the limited vasculature supply to the interior of the tumor, causing cells to proliferate at varying rates [114,115]. Many chemotherapeutic drugs are most effective on proliferating cells, allowing quiescent cells in the interior to evade treatment [116]. Hypoxia can also induce the activation of cell survival or pro-angiogenic genes, leading to certain populations of cells that become drug-resistant [117,118]. Additionally, drugs that rely on an oxygen-based free-radical mechanism cannot function on cells in a hypoxic (i.e. oxygen low) environment [119]. pH is also low in the extracellular space of hypoxic tumors, causing weakly basic drugs to become protonated, hindering their ability to cross the cell membrane [120].

Due to the lack of a fully functional vasculature system, fluid cannot be properly removed from the tumor through lymphatic vessels; therefore, IFP is often higher in tumors than in healthy tissues. This increased pressure inhibits the convection of macromolecules into the tissue [121,122]. Composition and organization of the tumor ECM can also determine whether drugs can penetrate into the tumor. For example, collagen, which is often upregulated in tumor microenvironments [63], has been shown to contribute to drug transport resistance in the interstitial space, possibly in conjunction with GAG binding [121]. *In vitro* studies of breast cancer cells in 3D collagen matrices revealed that cells in softer regions were more susceptible to paclitaxel treatment compared to those in stiffer regions, suggesting that tissue stiffness barriers can provide a degree of chemoprotection [77]. Organized protein structures and tightly packed tumors can also inhibit drug penetration [121,123]. Additionally, in certain types of mucinous cancers, an upregulation in mucin production provides individual cells with a physical barrier against drug treatment. This mucous layer makes the cells measurably stiffer and reduces the cytotoxic effects of applied chemotherapeutic agents [124].

3.2. Adhesion-conferred drug resistance

Adhesion to the ECM and other cells has been shown to confer chemoresistance to cancer cells through the activation of various pro-

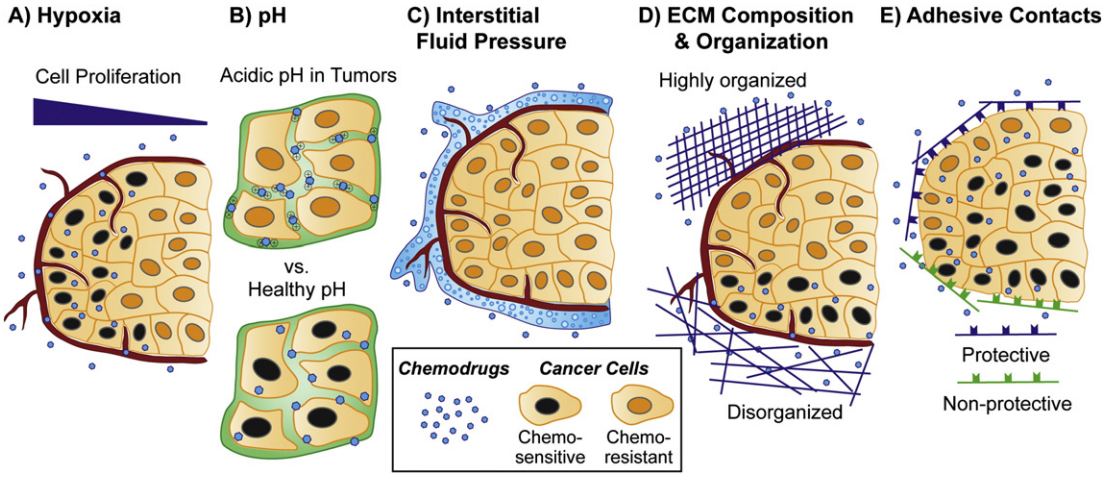

Fig. 2. *ECM-conferred barriers to treatment.* A) Hypoxic conditions due to poor vascularization of the tumor result in poor proliferation of cells in the interior. As many chemotherapuetic drugs target proliferating cells, more quiescent cells can survive treatment. B) Waste products from the rapidly dividing cancer cells contribute to low pH in the tumor microenvironment, which can protonate weakly basic drugs, preventing their passage through the cell membrane. C) Interstitial fluid pressure is higher in tumors, providing a physical barrier to drug diffusion. D) Tumor-directed, highly organized ECM structures inhibit drug penetration. E) The binding of certain integrins to the ECM conveys chemoresistance by activating pro-survival pathways.

survival pathways [13,125,126]. Cell survival pathways such as PI3K/Akt, p53 MAPK, ERK/MAPK, and Rho/ROCK have been shown to be activated upon binding to the ECM, and have thus become a major focus of anticancer therapies [127–131].

In multiple myeloma (MM), the malignant plasma cells in the bone marrow attach to stromal elements using several adhesion receptors, directly affecting their proliferation and survival, specifically in response to chemotherapeutic agents such as glucocorticoids (e.g. prednisone, dexamethasone), alkylating agents (melphalane), Velcade™, Thalidomide™ and imatinib mesylate (Gleevec™) [110,132–138]. Despite conventional high-dose therapies and novel treatments, MM remains an incurable disease as almost all patients relapse and develop some form of drug resistance [132,139]. Even in early stages, when the malignant cells may not harbor genetic lesions that could confer constitutive drug resistance, the epigenetic protection provided by the stromal microenvironment may enable some cells to survive within the bone marrow. This sub-clinical period may endure long enough for the development of additional genetic events that may be required for the emergence of clinical resistance and relapse of the MM tumor population after an apparent clinical remission. Integrin-mediated adhesion has been described in several MM cell lines *in vitro*, as well as in MM cells from patients. Both cultured MM cell lines and patient-derived MM cells adhere to vitronectin and fibronectin via the $\alpha_v\beta_3$ integrin, or to VCAM-1 via the $\alpha_4\beta_1$ integrin [140–142]. β_1 integrin-mediated adhesion of MM cells to fibronectin confers protection against drug-induced apoptosis, and triggers NFκB-dependent transcription and secretion of IL-6, a major MM growth and survival factor [143]. Additionally, hyaluronan, acting via the cell surface receptor CD44, has been shown to confer survival against IL-6 starvation or dexamethasone-induced apoptosis of human MM cells [144,145].

In breast cancer, clinical prognosis in patients is tightly linked to specific gene expression profiles, many of which are dominated by genes controlling ECM expression [146]. For instance, resistance to chemotherapeutic agents 5-fluorouracil, epirubicin, and cyclophosphamide are dependent on the protein composition of the stromal ECM [147]. Several studies have shown that BC cell adhesion to the ECM plays an important role in cancer cell survival post-chemotherapy treatment, identifying specific targets such as collagen [148,149], HA [150,151], and fibulin-1 [152]. An examination of many BC cells has indicated that they display diverse alterations in the expression of different integrins, including $\alpha_6\beta_4$, $\alpha_6\beta_1$, $\alpha_v\beta_3$, as well as the Thomsen–Friedenreich antigen (TF-Ag) and E-cadherin [107,108,153]. *In vitro*, 3D hydrogels containing laminin or fibronectin were found to convey chemoresistance to ERK inhibition or BRAF inhibition, respectively, further illustrating the importance of cell–ECM protective adhesion [62].

4. ECM-based cancer treatments

Most conventional chemotherapeutic interventions available today rely on inhibiting cancer cell proliferation. While this is an important aspect of the disease to target, most patients ultimately die from their metastases, not their primary tumors. Thus, there is a pressing need for novel interventions that can prevent the further dissemination of cancer cells after treatment. As ECM interactions play an important role in cancer cell survival and behavior, ECM-targeted therapies provide a promising approach to this need, either in preventing ECM-conferred chemoresistance or by altering the extracellular environment such that current therapies can overcome physical treatment limits. Many ECM-targeted chemotherapeutic drugs have been developed that attempt to inhibit specific integrin interactions, matrix metalloproteinase (MMP) activity, or the synthesis/degradation of the ECM, all of which will be described in the following sections.

4.1. Integrin-targeted drugs

Interventions that target specific integrin interactions aim at preventing protective downstream signaling effects conferred by the ligation of certain receptors (see Table 1). ATN-161 is a noncompetitive inhibitor of the fibronectin PHSRN sequence (the synergy sequence that enhances RGD binding) and is unique in that it does not block integrin-dependent adhesion, but rather targets downstream signaling via the $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins [154–156]. When used in combination with traditional chemotherapy, ATN-161 reduced tumor cell proliferation and improved survival in a colon cancer model due to its anti-angiogenic and anti-pro-survival properties [155]. In patients with advanced solid tumors, one third of patients exhibited prolonged stable disease [157], and further phase II studies are currently being performed [158]. Also targeting the $\alpha_v\beta_3$ integrin is Etaracizumab (Abergrin/MEDI-522, a variant of previous Vitaxin/MEDI-523), a humanized monoclonal antibody engineered from LM609. While preclinical studies for this drug were promising, phase II studies with patients exhibiting stage IV metastatic melanoma showed no clinical improvement [159].

Other integrin-targeted inhibitors have also attempted to target adhesions promoting tumor-induced angiogenesis and cell survival. First described by the Kessler group, Cilengitide, a cyclic-RGD pentapeptide, aimed at inhibiting invasive, pro-angiogenic endothelial cells from binding to the $\alpha_v\beta_3$ integrin, thereby inducing apoptosis and hindering cell migration [160]. In glioblastoma patients, this drug aimed to inhibit both $\alpha_v\beta_3$ and $\alpha_v\beta_5$, but after strong phase II trials, fell short in phase III

Table 1
Examples of integrin-targeted chemotherapeutic drugs.

Drug	Targeted integrin(s)	Clinical trial state[†]
Cilengitide	$\alpha_v\beta_5$ /$\alpha_v\beta_3$/$\alpha_5\beta_1$/$\alpha_{IIb}\beta_3$	Phase III
ATN-161	$\alpha_5\beta_1$/$\alpha_v\beta_3$	Phase II
Etaracizumab (Abegrin, MEDI-522)[*]	$\alpha_v\beta_3$	Phase II
Intetumumab (CNTO 95)	α_v	Phase II
Volociximab (M200)	$\alpha_5\beta_1$	Phase II
E7820	α_2	Phase II
17E6 (EMD 525797)	α_v	Phase II
Abituzumab (EMD 525797)	α_v	Phase I/II
SC-68448	$\alpha_v\beta_3$/$\alpha_{IIb}\beta_3$	Phase I
GLPG 0187	$\alpha_v\beta_1$/$\alpha_v\beta_3$/$\alpha_v\beta_5$/$\alpha_v\beta_6$/ $\alpha_5\beta_1$	Phase I
Abciximab (ReoPro, C7E3)[**] [207]	$\alpha_{IIb}\beta_3$/$\alpha_v\beta_3$/$\alpha_M\beta_2$	Pre-clinical
S137 and S247	$\alpha_v\beta_3$	Pre-clinical
SB 265123 [178]	$\alpha_v\beta_5$ /$\alpha_v\beta_3$	Pre-clinical
SM256 [208]	$\alpha_v\beta_3$	Pre-clinical
SD983 [208]	$\alpha_v\beta_3$	Pre-clinical
SCH221153 [209]	$\alpha_v\beta_5$ /$\alpha_v\beta_3$	Pre-clinical

References are indicated for drugs not mentioned in the text.
[*] Derived from Vitaxin/MEDI-523.
[**] Currently on the market for acute coronary syndrome.
[†] Ongoing, or last reported highest-level state.

[161]. Intetumumab (formerly CNTO 95), a human monoclonal antibody to the α_v integrin, unfortunately showed a decrease in both progression-free survival and overall survival in phase II trials of metastatic castration-resistance prostate cancer [162].

Still, there are multiple promising interventions currently undergoing clinical trials. Volociximab, an $\alpha_5\beta_1$ inhibiting antibody, has completed a phase Ib safety and pharmacokinetic study, in combination with carboplatin and paclitaxel, for advanced non-small-cell lung cancer [163]. An α_v-targeting monoclonal antibody, Abituzumab (EMD 525797), has also shown promise in a randomized phase I/II POSEIDON trial when combined with Cetuximab and Irinotecan in KRAS wild-type metastatic colorectal cancer [164]. While the progression-free survival target was not met, patients with tumors exhibiting high $\alpha_v\beta_6$ expression benefited from the treatment [164]. In preclinical trials, S137 and S247, small RGD-peptidomimetic antagonists, were able to inhibit lung metastasis in mice [12,165]. Phase I studies have been completed in patients with advanced malignancies using E7820, an anti-angiogenic oral inhibitor of integrin α_2 expression, and it is currently under investigation in phase II trials [166].

Human specific antibody 17E6 (EMD 525797) has been shown to block *in vivo* tumor growth of xenografts expressing $\alpha_v\beta_3$ in 6 different human melanomas and 5 carcinomas, but did not affect $\alpha_v\beta_3$-negative tumors. Furthermore, the effect was determined to be directly related to anti-tumor activity and not due to anti-angiogenic activity of α_v–integrin antagonists [167]. Currently, this drug is under investigation in phase II clinical trials [168]. Another oral compound in Phase I clinical trials is SC-68448, an RGD-peptidomimetic antagonist. In preclinical studies, this compound inhibited rat Leydig cell tumor growth in mice by up to 80% and completely blocked the development of hypercalcemia [169,170]. Another drug targeting RGD integrins is GLPG 0187, which inhibits $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$ and $\alpha_5\beta_1$, and is currently in phase Ib trials [171].

4.2. Drugs targeting ECM synthesis and degradation

Other ECM-based approaches include drugs targeting ECM synthesis via cytokine inhibitors (i.e. TGF-β inhibitors) and drugs targeting ECM degradation (i.e. MMP and proteinase inhibitors). TGF-β-targeting drugs hinder TGF-β-promoted secretion and processing of ECM proteins, such as collagen and fibronectin [172]. Fresolimumab (GC1008) is one such example; however, few clinical effects were seen in phase I trials [173–177]. As this subset of drugs is cytokine-based, the topic will not be covered here in detail, but can be found reviewed excellently elsewhere [178].

MMP inhibitors make up a large majority of ECM-targeted therapeutic approaches and are reviewed elsewhere [178]. Despite early failures [18], they remain a promising set of drugs due to their potential ability to hinder extravasation of tumor cells into different tissues during early stages of cancer progression. Endostar, a novel recombinant human endostatin, has shown promising results in a variety of trials. While the mechanism of action remains unclear, one study suggests that it acts by suppressing activity of MMPs-2 and -9 [179]. For the treatment of osteosarcoma patients, it showed an enhancement in event-free survival and decreased occurrence and progression of metastases [180]. For non-small-cell lung cancer in phase III trials, Endostar, in combination with platinum-based chemotherapy, resulted in significant clinical and survival benefits [181].

Heparanase, an enzyme responsible for degrading and remodeling the ECM, has been shown to be upregulated in multiple tumor types and in conjunction with distant metastases [182], making its inhibition an attractive target [61]. Adjuvant heparanase inhibitor PI-88 therapy for the treatment of hepatocellular carcinoma has shown positive results in phase II trials, with a 13% increase in recurrence-free rate and a postponement in time to recurrence of 78% [183]. Proteoglycans are also a potential target as these molecules can affect angiogenesis and cancer growth [184], and it has been shown that by inhibiting the glycosaminoglycan (GAG) component using 5-hexyl-2′-deoxyuridine (HUdR), GAG incorporation can be reduced. When intracardially transplanted tumor cells were treated with HUdR in mice, the number of metastatic nodules were decreased in an organ-specific manner [185].

4.3. Drugs targeting physical barriers to chemotherapy

As mentioned earlier, one major obstacle in effective drug delivery is the physical constraints imposed by the tumor microenvironment. Thus, combining cancer cell-killing drugs with ECM-degrading drugs could prove to be an effective strategy. In preclinical studies of high prostate PC3 tumors in mice, PEGPH20, a pegylated variant of the HA-degrading enzyme rHuPH20, when delivered in combination with chemotherapy (Docetaxel and Liposomal Doxorubicin), induced an anti-tumor response, inhibiting growth by 70% [186]. Effective ECM-remodeling was observed through decreased tumor IFP and water content, decompressed tumor vessels, and increased tumor vascular area, all of which likely enhance drug perfusion [186]. Other methods to reduce IFP, e.g. collagenase [187] and Paclitaxel pretreatment [188], have been explored, resulting in enhanced uptake of small molecules.

Hypoxia can be addressed with the use of nontoxic prodrugs that become activated under hypoxic conditions. One such example, Tirapazamine (SR-4233), a potent and selective killer of hypoxic cells [189], has been examined in combinatorial treatments. While earlier phase III trials showed promising results [190], a successive trial failed to produce positive outcomes [191]; confounding results could be due to the reduced ability of the drug to penetrate the tumor [192].

In order to overcome the acidic tumor microenvironment, raising the local pH with small molecules or creating pH-sensitive nanodrugs have been explored. By raising endosomal pH with various agents, uptake of the basic drugs doxorubicin and mitoxantrone is enhanced, improving their potential therapeutic effectiveness [193]. Ionizable pH-sensitive drug delivery systems, nanosystems containing acid-labile chemical bonds, and gas-generating pH-sensitive nanosystems are promising new techniques being explored to harness the inherent properties of the acidic interstitial space [194].

4.4. Matrix-based tumor immune therapy

One recent and exciting area of exploration has been the development of specific matrix molecules to target the activation of anti-

tumor immune cells in the tumor microenvironment. Adoptive cell therapy (ACT) aims to treat cancer with *ex vivo* generated T lymphocytes [195], and thus requires large numbers of activated T cells. *In vivo*, T cells are activated upon binding to specific ligands on the surface of antigen presenting cells [196]. To activate T cells *in vitro*, artificial antigen presentation has been developed [197]. A number of strategies to optimize this process exist, including identification of the required ligand density for T cell activation [198] and the presentation of anti-CD3 monoclonal antibodies in synthetic nanoarrays for control over T cell activation and subsequent expression of desired cellular responses [199]. Future research in this vein will focus on the optimization of high throughput T cell activation and expansion, most likely in parallel with microfluidic systems capable of mimicking the 3D interactions between T cells and synthetic antigen presenting cells [200], with the ultimate goal of treating cancer cells *in vivo* with T cells that have been prepared *ex vivo*. This strategy can be employed in conjunction with bioengineered growth factors that target the cancer ECM with superaffinity, allowing for more complete control over the regions to which cancer cells or immune cells attach [201].

5. Future outlook and conclusions

The phenomenon whereby malignant cells reach protective niches in the body and subsequently acquire resistance to chemotherapy via specific, adhesion-mediated signaling processes is still poorly characterized at the molecular level, but understanding this process could be the key to discovering why chemotherapy fails to provide a long-term cure in many cases. Due to the ubiquitous nature of the ECM in healthy tissue, it is of great importance to consider the specificity of ECM-targeted drugs, as off-target effects could prove detrimental to the body. Adverse bleeding events are associated with the use of $\alpha_{IIb}\beta_3$ antagonists, as they can also alter platelet response [202]. Therefore, the goal of systemic administration should be to enhance cancer cell-directed recognition.

The primary obstacle in interpreting the limited data available on adhesion-dependent drug resistance arises from the enormous molecular complexity of the cellular adhesive environment, making it very difficult to identify the specific epitopes responsible for protective effects. Moreover, studies in recent years have shown that adhesion-mediated signaling is affected not only by the chemical nature of the adhesive environment but also by multiple physical features of the matrix, including the spatial patterning of the adhesive epitopes available for cell binding, the topography of the surface and the rigidity of the substrate [203]. Additionally, as the tumor microenvironment has been put into the spotlight as a major mediator of cancer cell fate, the need for a proper 3D model has arisen, in which cell–cell, cell–matrix and organizational properties can all be precisely controlled. To do this, the ECM microenvironment for each diverse cancer type must be precisely determined. A novel approach aimed at controlling and defining the adhesive mechanisms underlying protective signaling and metastasis in cancer cells in 3D, and subsequently altering these adhesions to confer chemosensitivity to resistant cells, is of great importance and need.

New approaches to leveraging the relationship between cancer cells and the ECM have emerged very recently with the experimental implantation of cancer 'traps'—synthetic substrates designed to encourage cancer cells to enter but not leave [204]. These devices can be used as diagnostic tools to identify the presence of circulating tumor cells, but also have the potential for removal of invasive tumor cells from the body by simply out-'competing' tumor niches for circulating cancer cells.

Recent observations of the relationship between cell–ECM adhesion and malignant phenotypes suggest that simple, low-cost, label-free, image-analysis-based characterization of adhesion signatures may play a role in clinical diagnostics [205,206]. The continued development of 3D perfusion systems that accurately recapitulate the force basis of cancer cell/tumor ECM interactions will play a key role in streamlining the drug discovery process considering the sometimes major differences in drug efficacy on 2D and 3D substrates. As the transition to high throughput cell culture and image analysis techniques led to a new age in drug discovery, the development of high throughput 3D ECM systems in the coming decade will prove financially valuable by drastically lowering the rate of false positives. In many cases combinatorial drug treatments have been found to be more effective against cancer than single drugs, and thus eliminating false positives from these cocktails will only enhance the efficiency of combinatorial testing.

Acknowledgements

The authors would like to acknowledge the Max Planck Society for generous support in all respects.

References

[1] T. Helleday, E. Petermann, C. Lundin, B. Hodgson, R.A. Sharma, DNA repair pathways as targets for cancer therapy, Nat. Rev. Cancer 8 (2008) 193–204.
[2] A.D. Norden, J. Drappatz, P.Y. Wen, Antiangiogenic therapies for high-grade glioma, Nat. Rev. Neurol. 5 (2009) 610–620.
[3] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, Cell 126 (2006) 677–689.
[4] E. Cavalcantiadam, et al., Lateral spacing of integrin ligands influences cell spreading and focal adhesion assembly, Eur. J. Cell Biol. 85 (2006) 219–224.
[5] D.-H. Kim, P.P. Provenzano, C.L. Smith, A. Levchenko, Matrix nanotopography as a regulator of cell function, J. Cell Biol. 197 (2012) 351–360.
[6] A.L. Berrier, K.M. Yamada, K.M. Yamada, Cell–matrix adhesion, J. Cell. Physiol. 213 (2007) 565–573.
[7] R.O. Hynes, The extracellular matrix: not just pretty fibrils, Science 326 (2009) 1216–1219.
[8] M.A. Wozniak, K. Modzelewska, L. Kwong, P.J. Keely, Focal adhesion regulation of cell behavior, Biochim. Biophys. Acta (BBA) - Mol. Cell Res. 1692 (2004) 103–119.
[9] A. Pathak, S. Kumar, Independent regulation of tumor cell migration by matrix stiffness and confinement, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 10334–10339.
[10] A.L. Bauer, T.L. Jackson, Y. Jiang, Topography of extracellular matrix mediates vascular morphogenesis and migration speeds in angiogenesis, PLoS Comput. Biol. 5 (2009), e1000445.
[11] J. Kim, R.C. Hayward, Mimicking dynamic in vivo environments with stimuli-responsive materials for cell culture, Trends Biotechnol. 30 (2012) 426–439.
[12] K.E. Shannon, et al., Anti-metastatic properties of RGD-peptidomimetic agents S137 and S247, Clin. Exp. Metastasis 21 (2004) 129–138.
[13] T. Oskarsson, Extracellular matrix components in breast cancer progression and metastasis, Breast 22 (2013) S66–S72.
[14] M. Larsen, V.V. Artym, J.A. Green, K.M. Yamada, K.M. Yamada, The matrix reorganized: extracellular matrix remodeling and integrin signaling, Curr. Opin. Cell Biol. 18 (2006) 463–471.
[15] M. Allinen, et al., Molecular characterization of the tumor microenvironment in breast cancer, Cancer Cell 6 (2004) 17–32.
[16] D. Bourboulia, W.G. Stetler-Stevenson, Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs): positive and negative regulators in tumor cell adhesion, Semin. Cancer Biol. 20 (2010) 161–168.
[17] E. Hadler-Olsen, J.-O. Winberg, L. Uhlin-Hansen, Matrix metalloproteinases in cancer: their value as diagnostic and prognostic markers and therapeutic targets, Tumor Biol. 34 (2013) 2041–2051.
[18] L.M. Coussens, B. Fingleton, L.M. Matrisian, Matrix metalloproteinase inhibitors and cancer: trials and tribulations, Science 295 (2002) 2387–2392.
[19] J.R. Tse, A.J. Engler, Preparation of Hydrogel Substrates with Tunable Mechanical Properties, John Wiley & Sons, Inc, 2010 10.16.1–10.16.16, http://dx.doi.org/10.1002/0471143030.cb1016s47.
[20] N. Zaari, P. Rajagopalan, S.K. Kim, A.J. Engler, J.Y. Wong, Photopolymerization in microfluidic gradient generators: microscale control of substrate compliance to manipulate cell response, Adv. Mater. 16 (2004) 2133–2137.
[21] S.R. Peyton, A.J. Putnam, Extracellular matrix rigidity governs smooth muscle cell motility in a biphasic fashion, J. Cell. Physiol. 204 (2005) 198–209.
[22] L.G. Vincent, Y.S. Choi, B. Alonso-Latorre, J.C. del Álamo, A.J. Engler, Mesenchymal stem cell durotaxis depends on substrate stiffness gradient strength, Biotechnol. J. 8 (2013) 472–484.
[23] A.S. Rowlands, P.A. George, J.J. Cooper-White, Directing osteogenic and myogenic differentiation of MSCs: interplay of stiffness and adhesive ligand presentation, Am. J. Physiol. Cell Physiol. 295 (2008) C1037–C1044.
[24] N. Huebsch, et al., Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate, Nat. Mater. 9 (2010) 518–526.
[25] Y.-R.V. Shih, K.-F. Tseng, H.-Y. Lai, C.-H. Lin, O.K. Lee, Matrix stiffness regulation of integrin-mediated mechanotransduction during osteogenic differentiation of human mesenchymal stem cells, J. Bone Miner. Res. 26 (2011) 730–738.
[26] K. Ghosh, et al., Cell adaptation to a physiologically relevant ECM mimic with different viscoelastic properties, Biomaterials 28 (2007) 671–679.
[27] N.D. Evans, et al., Substrate stiffness affects early differentiation events in embryonic stem cells, Eur. Cell. Mater. 18 (2009) 1–13 (discussion 13–4).
[28] R.A. Marklein, J.A. Burdick, Spatially controlled hydrogel mechanics to modulate stem cell interactions, Soft Matter 6 (2010) 136–143.

[29] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, Cell 126 (2006) 677–689.
[30] K. Saha, et al., Substrate modulus directs neural stem cell behavior, Biophys. J. 95 (2008) 4426–4438.
[31] A.W. Holle, A.J. Engler, More than a feeling: discovering, understanding, and influencing mechanosensing pathways, Curr. Opin. Biotechnol. 22 (2011) 648–654.
[32] R.J. Pelham, Y.L. Wang, Cell locomotion and focal adhesions are regulated by the mechanical properties of the substrate, Biol. Bull. 194 (1998) 348–349 (discussion 349–50).
[33] M. Lekka, et al., Elasticity of normal and cancerous human bladder cells studied by scanning force microscopy, Eur. Biophys. J. 28 (1999) 312–316.
[34] S.E. Cross, Y.-S. Jin, J. Rao, J.K. Gimzewski, Nanomechanical analysis of cells from cancer patients, Nat. Nanotechnol. 2 (2007) 780–783.
[35] S.E. Cross, et al., AFM-based analysis of human metastatic cancer cells, Nanotechnology 19 (2008) 384003.
[36] A. Fuhrmann, et al., AFM stiffness nanotomography of normal, metaplastic and dysplastic human esophageal cells, Phys. Biol. 8 (2011) 015007.
[37] R.J. Paccione, et al., Keratin down-regulation in vimentin-positive cancer cells is reversible by vimentin RNA interference, which inhibits growth and motility, Mol. Cancer Ther. 7 (2008) 2894–2903.
[38] M. Beil, et al., Sphingosylphosphorylcholine regulates keratin network architecture and visco-elastic properties of human cancer cells, Nat. Cell Biol. 5 (2003) 803–811.
[39] T.A. Krouskop, T.M. Wheeler, F. Kallel, B.S. Garra, T. Hall, Elastic moduli of breast and prostate tissues under compression, Ultrason. Imaging 20 (1998) 260–274.
[40] D.B. Plewes, J. Bishop, A. Samani, J. Sciarretta, Visualization and quantification of breast cancer biomechanical properties with magnetic resonance elastography, Phys. Med. Biol. 45 (2000) 1591–1610.
[41] M.J. Paszek, V.M. Weaver, The tension mounts: mechanics meets morphogenesis and malignancy, J. Mammary Gland Biol. Neoplasia 9 (2004) 325–342.
[42] K.R. Levental, et al., Matrix crosslinking forces tumor progression by enhancing integrin signaling, Cell 139 (2009) 891–906.
[43] D.Y. Zhang, S.L. Friedman, Fibrosis-dependent mechanisms of hepatocarcinogenesis, Hepatology 56 (2012) 769–775.
[44] M.J. Paszek, et al., Tensional homeostasis and the malignant phenotype, Cancer Cell 8 (2005) 241–254.
[45] M. Milosevic, A. Fyles, D. Hedley, R. Hill, The human tumor microenvironment: invasive (needle) measurement of oxygen and interstitial fluid pressure, Semin. Radiat. Oncol. 14 (2004) 249–258.
[46] M. Fang, J. Yuan, C. Peng, Y. Li, Collagen as a double-edged sword in tumor progression, Tumor Biol. 35 (2013) 2871–2882.
[47] J.T. Erler, et al., Lysyl oxidase is essential for hypoxia-induced metastasis, Nature 440 (2006) 1222–1226.
[48] M.A. Wozniak, R. Desai, P.A. Solski, C.J. Der, P.J. Keely, ROCK-generated contractility regulates breast epithelial cell differentiation in response to the physical properties of a three-dimensional collagen matrix, J. Cell Biol. 163 (2003) 583–595.
[49] T.A. Ulrich, A. Jain, K. Tanner, J.L. MacKay, S. Kumar, Probing cellular mechanobiology in three-dimensional culture with collagen–agarose matrices, Biomaterials 31 (2010) 1875–1884.
[50] K. Wolf, et al., Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force, J. Cell Biol. 201 (2013) 1069–1084.
[51] T.A. Ulrich, E.M. de Juan Pardo, S. Kumar, The mechanical rigidity of the extracellular matrix regulates the structure, motility, and proliferation of glioma cells, Cancer Res. 69 (2009) 4167–4174.
[52] E.Y. Tokuda, J.L. Leight, K.S. Anseth, Modulation of matrix elasticity with PEG hydrogels to study melanoma drug responsiveness, Biomaterials 35 (2014) 4310–4318.
[53] P. Soman, et al., Cancer cell migration within 3D layer-by-layer microfabricated photocrosslinked PEG scaffolds with tunable stiffness, Biomaterials 33 (2012) 7064–7070.
[54] W. Zhang, D.S. Choi, Y.H. Nguyen, J. Chang, L. Qin, Studying cancer stem cell dynamics on PDMS surfaces for microfluidics device design, Sci. Rep. 3 (2013).
[55] I. Levental, et al., A simple indentation device for measuring micrometer-scale tissue stiffness, J. Phys. Condens. Matter 22 (2010) 194120.
[56] M. Arnold, et al., Activation of integrin function by nanopatterned adhesive interfaces, ChemPhysChem 5 (2004) 383–388.
[57] C.S. Chen, M. Mrksich, S. Huang, G.M. Whitesides, D.E. Ingber, Geometric control of cell life and death, Science 276 (1997) 1425–1428.
[58] G. Maheshwari, G. Brown, D.A. Lauffenburger, A. Wells, L.G. Griffith, Cell adhesion and motility depend on nanoscale RGD clustering, J. Cell Sci. 113 (Pt 10) (2000) 1677–1686.
[59] U. Hersel, C. Dahmen, H. Kessler, RGD modified polymers: biomaterials for stimulated cell adhesion and beyond, Biomaterials 24 (2003) 4385–4415.
[60] B. Jeschke, et al., RGD-peptides for tissue engineering of articular cartilage, Biomaterials 23 (2002) 3455–3463.
[61] R. Harisi, A. Jeney, Extracellular matrix as target for antitumor therapy, Onco Targets Ther. 1387 (2015)http://dx.doi.org/10.2147/OTT.S48883.
[62] B.H. Blehm, N. Jiang, Y. Kotobuki, K. Tanner, Deconstructing the role of the ECM microenvironment on drug efficacy targeting MAPK signaling in a pre-clinical platform for cutaneous melanoma, Biomaterials 56 (2015) 129–139.
[63] C.A. Sherman-Baust, et al., Remodeling of the extracellular matrix through overexpression of collagen VI contributes to cisplatin resistance in ovarian cancer cells, Cancer Cell 3 (2003) 377–386.
[64] R. Glass, M. Möller, J.P. Spatz, Block copolymer micelle nanolithography, Nanotechnology 14 (2003) 1153–1160.
[65] S. Kruss, L. Erpenbeck, M.P. Schön, J.P. Spatz, Circular, nanostructured and biofunctionalized hydrogel microchannels for dynamic cell adhesion studies, Lab Chip 12 (2012) 3285–3289.
[66] S. Krishnamoorthy, C. Hinderling, H. Heinzelmann, Nanoscale patterning with block copolymers, Mater. Today 9 (2006) 40–47.
[67] S.V. Graeter, et al., Mimicking cellular environments by nanostructured soft interfaces, Nano Lett. 7 (2007) 1413–1418.
[68] T. Shahal, B. Geiger, I.E. Dunlop, J.P. Spatz, Regulation of integrin adhesions by varying the density of substrate-bound epidermal growth factor, Biointerphases 7 (2012) 23-11.
[69] G. Benton, E. Crooke, J. George, Laminin-1 induces E-cadherin expression in 3-dimensional cultured breast cancer cells by inhibiting DNA methyltransferase 1 and reversing promoter methylation status, FASEB J. 23 (2009) 3884–3895.
[70] Y. Teng, J. Qiu, Y. Zheng, X. Luo, L. Zhang, Effects of type I collagen and fibronectin on regulation of breast cancer cell biological and biomechanical characteristics, J. Med. Biol. Eng. 34 (2014) 62–68.
[71] L.E. Barney, et al., A cell–ECM screening method to predict breast cancer metastasis, Integr. Biol. 7 (2015) 198–212.
[72] G. Benton, H.K. Kleinman, J. George, I. Arnaoutova, Multiple uses of basement membrane-like matrix (BME/Matrigel) in vitro and in vivo with cancer cells, Int. J. Cancer 128 (2011) 1751–1757.
[73] A. Albini, et al., A rapid in vitro assay for quantitating the invasive potential of tumor cells, Cancer Res. 47 (1987) 3239–3245.
[74] P.A. Kenny, et al., The morphologies of breast cancer cell lines in three-dimensional assays correlate with their profiles of gene expression, Mol. Oncol. 1 (2007) 84–96.
[75] G. Benton, I. Arnaoutova, J. George, H.K. Kleinman, J. Koblinski, Matrigel: from discovery and ECM mimicry to assays and models for cancer research, Adv. Drug Deliv. Rev. 79–80 (2014) 3–18.
[76] J. Han, et al., Molecular predictors of 3D morphogenesis by breast cancer cell lines in 3D culture, PLoS Comput. Biol. 6 (2010), e1000684.
[77] C.R.I. Lam, et al., A 3D biomimetic model of tissue stiffness interface for cancer drug testing, Mol. Pharm. 11 (2014) 2016–2021.
[78] C.G. Rolli, T. Seufferlein, R. Kemkemer, J.P. Spatz, Impact of tumor cell cytoskeleton organization on invasiveness and migration: a microchannel-based approach, PLoS ONE 5 (2010), e8726.
[79] P.H.S. Tan, K.Z. Aung, S.L. Toh, J.C.H. Goh, S.S. Nathan, Three-dimensional porous silk tumor constructs in the approximation of in vivo osteosarcoma physiology, Biomaterials (2011)http://dx.doi.org/10.1016/j.biomaterials.2011.04.084.
[80] S. Corall, et al., \upalpha 5\upbeta 1-integrin and MT1-MMP promote tumor cell migration in 2D but not in 3D fibronectin microenvironments, Comput. Mech. 53 (2014) 499–510.
[81] A.D. Rape, S. Kumar, A composite hydrogel platform for the dissection of tumor cell migration at tissue interfaces, Biomaterials 35 (2014) 8846–8853.
[82] S.A. Biela, Y. Su, J.P. Spatz, R. Kemkemer, Different sensitivity of human endothelial cells, smooth muscle cells and fibroblasts to topography in the nano–micro range, Acta Biomater. 5 (2009) 2460–2466.
[83] W.A. Loesberg, X.F. Walboomers, J.J.W.A. van Loon, J.A. Jansen, The effect of combined hypergravity and micro-grooved surface topography on the behaviour of fibroblasts. - PubMed - NCBI, Cell Motil. Cytoskeleton 63 (2006) 384–394.
[84] M. Sidani, J. Wyckoff, C. Xue, J.E. Segall, J. Condeelis, Probing the microenvironment of mammary tumors using multiphoton microscopy, J. Mammary Gland Biol. Neoplasia 11 (2006) 151–163.
[85] P.P. Provenzano, et al., Collagen density promotes mammary tumor initiation and progression, BMC Med. 6 (2008) 11.
[86] P.P. Provenzano, D.R. Inman, K.W. Eliceiri, S.M. Trier, P.J. Keely, Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization, Biophys. J. 95 (2008) 5374–5384.
[87] R.J. Petrie, A.D. Doyle, K.M. Yamada, Random versus directionally persistent cell migration, Nat. Rev. Mol. Cell Biol. 10 (2009) 538–549.
[88] K. Wolf, P. Friedl, Extracellular matrix determinants of proteolytic and non-proteolytic cell migration, Trends Cell Biol. 21 (2011) 736–744.
[89] F. Sabeh, R. Shimizu-Hirota, S.J. Weiss, Protease-dependent versus -independent cancer cell invasion programs: three-dimensional amoeboid movement revisited, J. Cell Biol. 185 (2009) 11–19.
[90] K. Wolf, et al., Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion, Nat. Cell Biol. 9 (2007) 893–904.
[91] M. Milosevic, et al., Interstitial fluid pressure predicts survival in patients with cervix cancer independent of clinical prognostic factors and tumor oxygen measurements, Cancer Res. 61 (2001) 6400–6405.
[92] S.S. Nathan, et al., Elevated physiologic tumor pressure promotes proliferation and chemosensitivity in human osteosarcoma, Clin. Cancer Res. 11 (2005) 2389–2397.
[93] K.Z. Aung, B.P. Pereira, P.H.S. Tan, H.C. Han, S.S. Nathan, Interstitial fluid pressure as an alternate regulator of angiogenesis independent of hypoxia driven HIF-1α in solid tumors, J. Orthop. Res. 30 (2012) 2038–2045.
[94] S.S. Nathan, et al., Tumor interstitial fluid pressure may regulate angiogenic factors in osteosarcoma, J. Orthop. Res. 26 (2008) 1520–1525.
[95] I.A. Khawar, J.H. Kim, H.-J. Kuh, Improving drug delivery to solid tumors: priming the tumor microenvironment, J. Control. Release 201 (2015) 78–89.
[96] A. Berg, A.-K.H. Ekwall, K. Rubin, J. Stjernschantz, R.K. Reed, Effect of PGE1, PGI2, and PGF2α analogs on collagen gel compaction in vitro and interstitial pressure in vivo, Am. J. Physiol. Heart Circ. Physiol. 274 (1998) H663–H671.
[97] M.J. Mitchell, M.R. King, Computational and experimental models of cancer cell response to fluid shear stress, Front. Oncol. 3 (2013).
[98] J. Kim, W. Yu, K. Kovalski, L. Ossowski, Requirement for specific proteases in cancer cell intravasation as revealed by a novel semiquantitative PCR-based assay, Cell 94 (1998) 353–362.

[99] M. Bockhorn, R.K. Jain, L.L. Munn, Active versus passive mechanisms in metastasis: do cancer cells crawl into vessels, or are they pushed? Lancet Oncol. 8 (2007) 444–448.
[100] W.J. Polacheck, J.L. Charest, R.D. Kamm, Interstitial flow influences direction of tumor cell migration through competing mechanisms, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 11115–11120.
[101] H. Qazi, Z.-D. Shi, J.M. Tarbell, Fluid shear stress regulates the invasive potential of glioma cells via modulation of migratory activity and matrix metalloproteinase expression, PLoS ONE 6 (2011), e20348.
[102] M.J. Mitchell, M.R. King, Fluid shear stress sensitizes cancer cells to receptor-mediated apoptosis via trimeric death receptors, New J. Phys. 15 (2013) 015008.
[103] K. Ghosh, et al., Tumor-derived endothelial cells exhibit aberrant Rho-mediated mechanosensing and abnormal angiogenesis in vitro, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 11305–11310.
[104] O. Trédan, C.M. Galmarini, K. Patel, I.F. Tannock, Drug resistance and the solid tumor microenvironment, J. Natl. Cancer Inst. 99 (2007) 1441–1454.
[105] C.S. Mitsiades, N.S. Mitsiades, N.C. Munshi, P.G. Richardson, K.C. Anderson, The role of the bone microenvironment in the pathophysiology and therapeutic management of multiple myeloma: interplay of growth factors, their receptors and stromal interactions, Eur. J. Cancer 42 (2006) 1564–1573.
[106] T. Vincent, N. Mechti, Extracellular matrix in bone marrow can mediate drug resistance in myeloma, Leuk. Lymphoma 46 (2015) 803–811.
[107] E.K. Sloan, et al., Tumor-specific expression of αvβ3 integrin promotes spontaneous metastasis of breast cancer to bone, Breast Cancer Res. 8 (2006) R20.
[108] M.A. Chrenek, P. Wong, V.M. Weaver, Tumour-stromal interactions. Integrins and cell adhesions as modulators of mammary cell survival and transformation, Breast Cancer Res. 3 (2001) 224–229.
[109] P.J. Morin, in: B. Teicher (Ed.), Cancer Drug Resistance, Humana Press 2006, pp. 201–210, http://dx.doi.org/10.1007/978-1-59745-035-5_11.
[110] J.S. Damiano, W.S. Dalton, Integrin-mediated drug resistance in multiple myeloma, Leuk. Lymphoma 38 (2000) 71–81.
[111] R.K. Jain, Transport of Molecules, Particles, and Cells in Solid Tumors, 1 (2003) 241–263, http://dx.doi.org/10.1146/annurev.bioeng.1.1.241.
[112] S.H. Jang, M.G. Wientjes, D. Lu, J.L.S. Au, Drug delivery and transport to solid tumors, Pharm. Res. 20 (2003) 1337–1350.
[113] A.H. Kyle, L.A. Huxham, D.M. Yeoman, A.I. Minchinton, Limited tissue penetration of taxanes: a mechanism for resistance in solid tumors, Clin. Cancer Res. 13 (2007) 2804–2810.
[114] I.F. Tannock, The relation between cell proliferation and the vascular system in a transplanted mouse mammary tumour, Br. J. Cancer 22 (1968) 258–273.
[115] A.S.E. Ljungkvist, et al., Vascular architecture, hypoxia, and proliferation in first-generation xenografts of human head-and-neck squamous cell carcinomas, Int. J. Radiat. Oncol. Biol. Phys. 54 (2002) 215–228.
[116] I. Tannock, Cell kinetics and chemotherapy: a critical review, Cancer Treat. Rep. 62 (1978) 1117–1133.
[117] T.G. Graeber, et al., Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours, 379 (1996) 88–91, http://dx.doi.org/10.1038/379088a0 (Published online: 04 January 1996).
[118] M. Kinoshita, D.L. Johnson, C.H. Shatney, Y.L. Lee, H. Mochizuki, Cancer cells surviving hypoxia obtain hypoxia resistance and maintain anti-apoptotic potential under reoxygenation, Int. J. Cancer 91 (2001) 322–326.
[119] P. Kovacic, J.A. Osuna Jr., Mechanisms of Anti-Cancer Agents Emphasis on Oxidative Stress and Electron Transfer, 2000.
[120] L.E. Gerweck, S. Vijayappa, S. Kozin, Tumor pH controls the in vivo efficacy of weak acid and base chemotherapeutics, Mol. Cancer Ther. 5 (2006) 1275–1279.
[121] P.A. Netti, D.A. Berk, M.A. Swartz, A.J. Grodzinsky, R.K. Jain, Role of extracellular matrix assembly in interstitial transport in solid tumors, Cancer Res. 60 (2000) 2497–2503.
[122] C.-H. Heldin, K. Rubin, K. Pietras, A. Östman, High interstitial fluid pressure ||[mdash]|| an obstacle in cancer therapy, Nat. Rev. Cancer 4 (2004) 806–813.
[123] R. Grantab, S. Sivananthan, I.F. Tannock, The penetration of anticancer drugs through tumor tissue as a function of cellular adhesion and packing density of tumor cells, Cancer Res. 66 (2006) 1033–1039.
[124] X. Wang, A.A. Shah, R.B. Campbell, K.-T. Wan, Glycoprotein mucin molecular brush on cancer cell surface acting as mechanical barrier against drug delivery, Appl. Phys. Lett. 97 (2010) 263703.
[125] G.V. Glinsky, Anti-adhesion cancer therapy, Cancer Metastasis Rev. 17 (1998) 177–185.
[126] R.L. Juliano, J.A. Varner, Adhesion molecules in cancer: the role of integrins, Curr. Opin. Cell Biol. 5 (1993) 812–818.
[127] N.O. Carragher, M.C. Frame, Focal adhesion and actin dynamics: a place where kinases and proteases meet to promote invasion, Trends Cell Biol. 14 (2004) 241–249.
[128] J. Zhao, J.-L. Guan, Signal transduction by focal adhesion kinase in cancer, Cancer Metastasis Rev. 28 (2009) 35–49.
[129] X. Zhao, J.-L. Guan, Focal adhesion kinase and its signaling pathways in cell migration and angiogenesis, Adv. Drug Deliv. Rev. 63 (2011) 610–615.
[130] K. Bommert, R.C. Bargou, T. Stühmer, Signalling and survival pathways in multiple myeloma, Eur. J. Cancer 42 (2006) 1574–1580.
[131] D.D. Schlaepfer, C.R. Hauck, D.J. Sieg, Signaling through focal adhesion kinase, Prog. Biophys. Mol. Biol. 71 (1999) 435–478.
[132] J.S. Damiano, A.E. Cress, L.A. Hazlehurst, A.A. Shtil, W.S. Dalton, Cell Adhesion Mediated Drug Resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines, Blood 93 (1999) 1658–1667.
[133] F. Sanz-Rodriguez, Chemokine stromal cell-derived factor-1alpha modulates VLA-4 integrin-mediated multiple myeloma cell adhesion to CS-1/fibronectin and VCAM-1, Blood 97 (2001) 346–351.
[134] D. Chauhan, et al., The bortezomib/proteasome inhibitor PS-341 and triterpenoid CDDO-Im induce synergistic anti-multiple myeloma (MM) activity and overcome bortezomib resistance, Blood 103 (2004) 3158–3166.
[135] R.C. Kane, P.F. Bross, A.T. Farrell, R. Pazdur, Velcade ®: U.S. FDA approval for the treatment of multiple myeloma progressing on prior therapy, Oncologist 8 (2003) 508–513.
[136] H.H. Yang, Overcoming drug resistance in multiple myeloma: the emergence of therapeutic approaches to induce apoptosis, J. Clin. Oncol. 21 (2003) 4239–4247.
[137] M. Azam, R.R. Latek, G.Q. Daley, Mechanisms of autoinhibition and STI-571/imatinib resistance revealed by mutagenesis of, Cell 112 (2003) 831–843.
[138] B.G.M. Durie, et al., Myeloma management guidelines: a consensus report from the Scientific Advisors of the International Myeloma Foundation, Hematol. J. 4 (2003) 379–398.
[139] D. Grossman, D.C. Altieri, Drug resistance in melanoma: mechanisms, apoptosis, and new potential therapeutic targets, Cancer Metastasis Rev. 20 (2001) 3–11.
[140] R. Ria, et al., αvβ3 integrin engagement enhances cell invasiveness in human multiple myeloma, Haematologica 87 (2002) 836–845.
[141] F. Sanz-Rodriguez, N. Ruiz-Velasco, D. Pascual-Salcedo, J. Teixidó, Characterization of VLA-4-dependent myeloma cell adhesion to fibronectin and VCAM-1, Br. J. Haematol. 107 (1999) 825–834.
[142] T. Michigami, et al., Cell–cell contact between marrow stromal cells and myeloma cells via VCAM-1 and α4β1-integrin enhances production of osteoclast-stimulating activity, Blood 96 (2000) 1953–1960.
[143] H. Uchiyama, B.A. Barut, A.F. Mohrbacher, D. Chauhan, K.C. Anderson, Adhesion of human myeloma-derived cell lines to bone marrow stromal cells stimulates interleukin-6 secretion, Blood 82 (1993) 3712–3720.
[144] T. Vincent, M. Jourdan, M.S. Sy, B. Klein, N. Mechti, Hyaluronic acid induces survival and proliferation of human myeloma cells through an interleukin-6-mediated pathway involving the phosphorylation of retinoblastoma protein, J. Biol. Chem. 276 (2001) 14728–14736.
[145] M.V. Driel, et al., CD44 variant isoforms are involved in plasma cell adhesion to bone marrow stromal cells, Leukemia 16 (2002) 135–143.
[146] A. Bergamaschi, et al., Extracellular matrix signature identifies breast cancer subgroups with different clinical outcome, J. Pathol. 214 (2007) 357–367.
[147] P. Farmer, et al., A stroma-related gene signature predicts resistance to neoadjuvant chemotherapy in breast cancer, Nat. Med. 15 (2009) 68–74.
[148] W. Zuo, et al., Down-regulation of osteopontin expression by RNA interference affects cell proliferation and chemotherapy sensitivity of breast cancer MDA-MB-231 cells, Mol. Med. Rep. 5 (2011) 373–376.
[149] G. Said, et al., Extracellular matrix proteins modulate antimigratory and apoptotic effects of Doxorubicin. - PubMed - NCBI, Chemother. Res. Pract. 2012 (2012) 1–10.
[150] S. Misra, S. Ghatak, B.P. Toole, Regulation of MDR1 expression and drug resistance by a positive feedback loop involving hyaluronan, phosphoinositide 3-kinase, and ErbB2, J. Biol. Chem. 280 (2005) 20310–20315.
[151] S. Misra, S. Misra, S. Ghatak, A. Zoltan-Jones, B.P. Toole, Regulation of multidrug resistance in cancer cells by hyaluronan, J. Biol. Chem. 278 (2003) 25285–25288.
[152] S.M. Pupa, et al., Regulation of breast cancer response to chemotherapy by fibulin-1, Cancer Res. 67 (2007) 4271–4277.
[153] G. Berx, G. Berx, F. Van Roy, F. Van Roy, The E-cadherin/catenin complex: an important gatekeeper in breast cancer tumorigenesis and malignant progression, Breast Cancer Res. 3 (2001) 289–293.
[154] D.L. Livant, et al., Anti-invasive, antitumorigenic, and antimetastatic activities of the PHSCN sequence in prostate carcinoma, Cancer Res. 60 (2000) 309–320.
[155] O. Stoeltzing, et al., Inhibition of integrin α5β1 function with a small peptide (ATN-161) plus continuous 5-FU infusion reduces colorectal liver metastases and improves survival in mice, Int. J. Cancer 104 (2003) 496–503.
[156] P. Khalili, et al., A non-RGD-based integrin binding peptide (ATN-161) blocks breast cancer growth and metastasis in vivo, Mol. Cancer Ther. 5 (2006) 2271–2280.
[157] M.E. Cianfrocca, et al., Phase 1 trial of the antiangiogenic peptide ATN-161 (Ac-PHSCN-NH2), a beta integrin antagonist, in patients with solid tumours, Br. J. Cancer 94 (2006) 1621–1626.
[158] F. Doñate, et al., Pharmacology of the novel antiangiogenic peptide ATN-161 (Ac-PHSCN-NH2): observation of a U-shaped dose-response curve in several preclinical models of angiogenesis and tumor growth, Clin. Cancer Res. 14 (2008) 2137–2144.
[159] P. Hersey, et al., A randomized phase 2 study of etaracizumab, a monoclonal antibody against integrin αvβ3, ± dacarbazine in patients with stage IV metastatic melanoma, Cancer 116 (2010) 1526–1534.
[160] M.A. Dechantsreiter, et al., N-methylated cyclic RGD peptides as highly active and selective α Vβ 3integrin antagonists, J. Med. Chem. 42 (1999) 3033–3040.
[161] R. Stupp, et al., Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial, Lancet Oncol. 15 (2014) 1100–1108.
[162] A. Heidenreich, et al., A randomized, double-blind, multicenter, phase 2 study of a human monoclonal antibody to human αν integrins (intetumumab) in combination with docetaxel and prednisone for the first-line treatment of patients with metastatic castration-resistant prostate cancer, Ann. Oncol. 24 (2013) 329–336.
[163] B. Besse, et al., Phase Ib safety and pharmacokinetic study of volociximab, an anti-α5β1 integrin antibody, in combination with carboplatin and paclitaxel in advanced non-small-cell lung cancer, Ann. Oncol. 24 (2012) mds281–mds296.
[164] E. Élez, et al., Abituzumab combined with cetuximab plus irinotecan versus cetuximab plus irinotecan alone for patients with KRAS wild-type metastatic colorectal cancer: the randomised phase I/II POSEIDON trial, Ann. Oncol. 26 (2015) 132–140.

[165] N. Reinmuth, et al., Alphavbeta3 integrin antagonist S247 decreases colon cancer metastasis and angiogenesis and improves survival in mice, Cancer Res. 63 (2003) 2079–2087.
[166] M. Mita, et al., Phase I study of E7820, an oral inhibitor of integrin alpha-2 expression with antiangiogenic properties, in patients with advanced malignancies, Clin. Cancer Res. 17 (2011) 193–200.
[167] F. Mitjans, et al., In vivo therapy of malignant melanoma by means of antagonists of αv integrins, Int. J. Cancer 87 (2000) 716–723.
[168] B. Mahalingam, et al., Atomic basis for the species-specific inhibition of αV integrins by monoclonal antibody 17E6 is revealed by the crystal structure of αVβ3 ectodomain-17E6 Fab complex, J. Biol. Chem. 289 (2014) 13801–13809.
[169] C.P. Carron, et al., A peptidomimetic antagonist of the integrin alpha(v)beta3 inhibits Leydig cell tumor growth and the development of hypercalcemia of malignancy, Cancer Res. 58 (1998) 1930–1935.
[170] C. Kumar, Integrin αvβ3 as a therapeutic target for blocking tumor-induced angiogenesis, Curr. Drug Targets 4 (2003) 123–131.
[171] G. Lorenzon, et al., Abstract 1568: GLPG0187, a small molecule integrin antagonist, shows good safety and decrease in CTX levels in single ascending dose study, Cancer Res. 70 (2014) 1568-1568.
[172] A.B. ROBERTS, U.I. HEINE, K.C. Flanders, M.B. SPORN, Transforming growth factor-β, Ann. N. Y. Acad. Sci. 580 (1990) 225–232.
[173] L.M. Rice, et al., Fresolimumab treatment decreases biomarkers and improves clinical symptoms in systemic sclerosis patients, J. Clin. Invest. 125 (2015) 2795–2807.
[174] H. Trachtman, et al., A phase 1, single-dose study of fresolimumab, an anti-TGF-|[beta]| antibody, in treatment-resistant primary focal segmental glomerulosclerosis, Kidney Int. 79 (2011) 1236–1243.
[175] J.C. Morris, et al., Phase I study of GC1008 (Fresolimumab): A human anti-transforming growth factor-beta (TGFβ) monoclonal antibody in patients with advanced malignant melanoma or renal cell carcinoma, PLoS ONE 9 (2014), e90353.
[176] J.P. Stevenson, et al., Immunological effects of the TGFβ-blocking antibody GC1008 in malignant pleural mesothelioma patients, OncoImmunology 2 (2013), e26218.
[177] C. Grütter, et al., A cytokine-neutralizing antibody as a structural mimetic of 2 receptor interactions, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 20251–20256.
[178] G.F. Weber, Why does cancer therapy lack effective anti-metastasis drugs? Cancer Lett. 328 (2013) 207–211.
[179] N. Lu, et al., Endostar suppresses invasion through downregulating the expression of matrix metalloproteinase-2/9 in MDA-MB-435 human breast cancer cells, Exp. Biol. Med. (Maywood) 233 (2008) 1013–1020.
[180] M. Xu, et al., Effects of endostar combined multidrug chemotherapy in osteosarcoma, Bone 57 (2013) 111–115.
[181] Y. Sun, et al., Long-term results of a randomized, double-blind, and placebo-controlled phase III trial: Endostar (rh-endostatin) versus placebo in combination with vinorelbine and cisplatin in advanced non-small cell lung cancer, Thorac. Cancer 4 (2013) 440–448.
[182] Y. Nadir, B. Brenner, Heparanase multiple effects in cancer, Thromb. Res. 133 (2014) S90–S94.
[183] C.-J. Liu, et al., Adjuvant heparanase inhibitor PI-88 therapy for hepatocellular carcinoma recurrence, World J. Gastroenterol. 20 (2014) 11384–11393.
[184] R.V. Iozzo, R.D. Sanderson, Proteoglycans in cancer biology, tumour microenvironment and angiogenesis, J. Cell. Mol. Med. 15 (2011) 1013–1031.
[185] A. Jeney, et al., Glycosaminoglycans as novel target in antitumor therapy, Tokai J. Exp. Clin. Med. 15 (1990) 167–177.
[186] C.B. Thompson, et al., Enzymatic depletion of tumor hyaluronan induces antitumor responses in preclinical animal models, Mol. Cancer Ther. 9 (2010) 3052–3064.
[187] L. Eikenes, Ø.S. Bruland, C. Brekken, C. de L. Davies, Collagenase increases the transcapillary pressure gradient and improves the uptake and distribution of monoclonal antibodies in human osteosarcoma xenografts, Cancer Res. 64 (2004) 4768–4773.
[188] A.G. Taghian, et al., Paclitaxel decreases the interstitial fluid pressure and improves oxygenation in breast cancers in patients treated with neoadjuvant chemotherapy: clinical implications, J. Clin. Oncol. 23 (2005) 1951–1961.
[189] E.M. Zeman, J.M. Brown, M.J. Lemmon, V.K. Hirst, W.W. Lee, SR-4233: a new bioreductive agent with high selective toxicity for hypoxic mammalian cells, Int. J. Radiat. Oncol. Biol. Phys. 12 (1986) 1239–1242.
[190] J. von Pawel, et al., Tirapazamine plus cisplatin versus cisplatin in advanced non-small-cell lung cancer: A report of the international CATAPULT I study group. Cisplatin and Tirapazamine in Subjects with Advanced Previously Untreated Non-Small-Cell Lung Tumors, J. Clin. Oncol. 18 (2000) 1351–1359.
[191] S.K. Williamson, et al., Phase III trial of paclitaxel plus carboplatin with or without tirapazamine in advanced non-small-cell lung cancer: Southwest Oncology Group Trial S0003, J. Clin. Oncol. 23 (2005) 9097–9104.
[192] K.O. Hicks, F.B. Pruijn, J.R. Sturman, W.A. Denny, W.R. Wilson, Multicellular resistance to tirapazamine is due to restricted extravascular transport: a pharmacokinetic/pharmacodynamic study in HT29 multicellular layer cultures, Cancer Res. 63 (2003) 5970–5977.
[193] C.M. Lee, I.F. Tannock, Inhibition of endosomal sequestration of basic anticancer drugs: influence on cytotoxicity and tissue penetration, Br. J. Cancer 94 (2006) 863–869.
[194] J. Liu, et al., pH-Sensitive nano-systems for drug delivery in cancer therapy, Biotechnol. Adv. 32 (2014) 693–710.
[195] C.H. June, Adoptive T cell therapy for cancer in the clinic, J. Clin. Invest. 117 (2007) 1466–1476.
[196] R.N. Germain, MHC-dependent antigen processing and peptide presentation: Providing ligands for T lymphocyte activation, Cell 76 (1994) 287–299.
[197] I. Platzman, J.-W. Janiesch, J. Matić, J.P. Spatz, Artificial antigen-presenting interfaces in the service of immunology, Isr. J. Chem. 53 (2013) 655–669.
[198] J. Deeg, et al., T cell activation is determined by the number of presented antigens, Nano Lett. 13 (2013) 5619–5626.
[199] J. Matić, J. Deeg, A. Scheffold, I. Goldstein, J.P. Spatz, Fine tuning and efficient T cell activation with stimulatory aCD3 nanoarrays, Nano Lett. 13 (2013) 5090–5097.
[200] I. Platzman, J.-W. Janiesch, J.P. Spatz, Synthesis of nanostructured and biofunctionalized water-in-oil droplets as tools for homing T cells, J. Am. Chem. Soc. 135 (2013) 3339–3342.
[201] M.M. Martino, et al., Growth factors engineered for super-affinity to the extracellular matrix enhance tissue healing, Science 343 (2014) 885–888.
[202] M. Millard, S. Odde, N. Neamati, Integrin targeted therapeutics, Theranostics 1 (2011) 154–188.
[203] Y.L. Wang, D.E. Discher, Cell Mechanics-Book, Elsevier, 2007 (at http://www.worldcat.org/title/cell-mechanics/oclc/157028004).
[204] S.M. Azarin, et al., In vivo capture and label-free detection of early metastatic cells, Nat. Commun. 6 (2015) 8094.
[205] K. Klein, T. Maier, V.C. Hirschfeld-Warneken, J.P. Spatz, Marker-free phenotyping of tumor cells by fractal analysis of reflection interference contrast microscopy images, Nano Lett. 13 (2013) 5474–5479.
[206] K. Klein, C.E. Rommel, V.C. Hirschfeld-Warneken, J.P. Spatz, Cell membrane topology analysis by RICM enables marker-free adhesion strength quantification, Biointerphases 8 (2013) 28.
[207] M. Trikha, et al., Multiple roles for platelet GPIIb/IIIa and alphavbeta3 integrins in tumor growth, angiogenesis, and metastasis, Cancer Res. 62 (2002) 2824–2833.
[208] J.S. Kerr, et al., Novel small molecule alpha v integrin antagonists: comparative anti-cancer efficacy with known angiogenesis inhibitors, Anticancer Res. 19 (1999) 959–968.
[209] C.C. Kumar, et al., Inhibition of angiogenesis and tumor growth by SCH221153, a dual alpha(v)beta3 and alpha(v)beta5 integrin receptor antagonist, Cancer Res. 61 (2001) 2232–2238.