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A mechanopharmacology approach to overcome chemoresistance in pancreatic cancer

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is a highly chemoresistant malignancy. This chemoresistant phenotype has been historically associated with genetic factors. Major biomedical research efforts were concentrated that resulted in the identification of subtypes characterized by specific genetic lesions and gene expression signatures that suggest important biological differences. However, to date, these distinct differences could not be exploited for therapeutic interventions. Apart from these genetic factors, desmoplasia and tumor microenvironment have been recognized as key contributors to PDAC chemoresistance. However, while several strategies targeting tumor-stroma have been explored including drugs against members of the Hedgehog family, they failed to meet the expectations in the clinical setting. These unsatisfactory clinical results suggest that, an important link between genetics and the influence of tumor microenvironment on PDAC chemoresistance remains to be elucidated. In this respect, mechanobiology is an emerging multidisciplinary field that encompasses cell and developmental biology as well as biophysics and bioengineering. Herein we provide a comprehensive overview of the key players in pancreatic cancer chemoresistance from the perspective of mechanobiology, and discuss novel experimental avenues such as elastic micropillar arrays that could provide fresh insights for the development of mechanobiology-targeted therapeutic approaches (know as mechanopharmacology) to overcome anticancer drug resistance in pancreatic cancer.

1. Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a devastating malignant disease, exhibiting one of the poorest prognoses of all solid tumors. With a 5-year survival of ~7%, PDAC is the 4th leading cause of cancer-death and is projected to be the second most lethal cancer by 2030 (Rahib et al., 2014; Siegel et al., 2017). This dismal trend is due to the rising incidence and poor outcome caused by lack of biomarkers for early screening/diagnosis, as well as poor efficacy of current treatments (Kleeff et al., 2016). The very aggressive nature and the early metastatic behavior of PDAC, frequently impede the potentially curative surgical resection. Even in the absence of metastasis, other pathological conditions, e.g. local infiltration of major retroperitoneal vessels, potentially exclude pancreatic resection (Paulson et al., 2013). Chemotherapy is therefore a crucial component in the treatment of unresectable (metastatic or locally-advanced) PDAC patients. However, the two most successful combination chemotherapeutic protocols [i.e. FOLFIRINOX (a combination of 5-fluorouracil (5-FU), leucovorin, irinotecan and oxaliplatin) and gemcitabine/nab-paclitaxel, resulted in modest survival benefits (<1year) which are unfortunately nullified by the significant untoward toxicity and a compromised quality of life for most PDAC patients (Conroy et al., 2011; Von Hoff et al., 2013). Despite concentrated efforts to extensively map the mutational landscape of PDAC, including the identification of specific subtypes (Bailey et al., 2016), and to better understand the molecular events underlying the initiation and progression of PDAC (Neesse et al., 2015), the molecular basis underlying the poor chemotherapeutic response remains elusive.

Over the past decade, the hypovascular and desmoplastic tumor

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microenvironment in PDAC has been recognized as the key determinant promoting both carcinogenesis and tumor progression as well as a leading mediator of chemoresistance. Hence, novel targeting strategies of various cellular/non-cellular stromal components and pathways were considered a promising approach to enhance the therapeutic efficacy (Neesse et al., 2015). However, none of these genuine efforts conducted in rigorous clinical phase II and III trials, met the clinical expectations and failed to lead to approved PDAC therapies (Bramhall et al., 2001; Bramhall et al., 2002; Moore et al., 2003). Additionally, recent experimental evidence has shown that tumor-associated fibroblasts may suppress, rather than promote, tumor growth. This highly controversial and open debate regarding whether or not the tumor stroma of PDAC is a 'friend or a foe', reinforces the need to critically re-evaluate the complexity of tumor-stroma interactions (Gore and Korc, 2014).

In the present review, we introduce the current knowledge regarding PDAC chemoresistance and the unsuccessful (pre)-clinical attempts to enhance the response to chemotherapeutics used in the clinical routine. From a mechanobiology perspective, elucidating the bidirectional interplay between drug action/resistance and mechanics, under the context of the highly genomically unstable landscape of PDAC, could represent the key to improve the yet unsatisfactory therapies targeting the hallmarks of PDAC including desmoplasia, inflammation, and immune suppression. Mechanobiology is an emerging multidisciplinary field which encompasses cell and developmental biology, bioengineering and biophysics; specifically, mechanobiology studies the impact of physical forces and the mechanical properties of the extracellular matrix (ECM) on cell behavior, cell/tissue morphogenesis and diseases that are highly regulated by pathological processes such as cancer (Jansen et al., 2015). We here adopted the term 'mechanopharmacology' that has been recently introduced by Krishnan and colleagues to define a new and wider conceptual field, that aims at investigating the impact of cell and tissue mechanics on pharmacological responsiveness, and its application to mechanistic investigations and drug screening (Krishnan et al., 2016). It is our strong belief that mechanopharmacology could be successful at the discovery of novel drug targets and antitumor agents to combat PDAC, in addition to explaining the basis for the modest survival benefits of existing therapies. Lastly, we provide examples of powerful mechanobiology tools that, in combination with high resolution light microscopy, pave the way to study with unprecedented detail, how cells apply forces, alter their microenvironment ('inside-out signaling') and, *vice versa*, how cells probe the mechanical properties of their microenvironment and translate this information together with the information obtained from other signals such as growth factors into a concerted response ('outside-in coupling').

2. Chemoresistance of PDAC

The prominent chemoresistant nature of PDAC appears to be multifactorial. In fact, various studies with different tumors of distinct cell lineage have shown that anticancer drug resistance is multifactorial (Shibue and Weinberg, 2017; Gonen and Assaraf, 2016; Zhitomirsky and Assaraf, 2016; Li et al., 2017; Wijdeven et al., 2016). The proposed molecular mechanisms responsible for this multidrug resistance, range from tumor cell-intrinsic mechanisms such as activation of anti-apoptotic signaling pathways, to extrinsic mechanisms including unique properties of the tumor microenvironment modulating drug uptake and activating escape pathways.

First and foremost, PDAC patients frequently display hypovascularity that, in conjunction to the extensive desmoplastic reaction, hampers effective drug delivery to tumor cells (Chu et al., 2007; Neesse et al., 2014). This physical barrier and related pathways will be the main focus of the following sections in the present review. PDAC cells often have dysregulated cellular transporters that can compromise or abolish the uptake of chemotherapeutic agents by these tumor cells. The most studied biomarker for drug activity/resistance in PDAC is the human equilibrative nucleoside transporter 1 (hENT1) which is the primary transporter that facilitates bidirectional transport of pyrimidine nucleosides and their analogues including gemcitabine, into cancer cells (Spratlin et al., 2004). Several clinical reports have indicated that the mRNA and protein levels of hENT1 is a predictive biomarker for PDAC patients treated with gemcitabine (Nordh et al., 2014). Specifically, the retrospective analysis of the phase III trials RTOG-9704 and ESPAC-1/3 showed that the overall survival (OS) was significantly longer in patients treated with gemcitabine with high-hENT1 expression. Notably, these OS benefits were not found in patients treated with 5-FU (Farrell et al., 2009; Greenhalf et al., 2014), implying a more predictive value than a prognostic role. However, the first biomarker-stratified trial (LEAP) with prospective analysis of (low) hENT1 expression comparing CO-1.01 with gemcitabine failed to validate this correlation in metastatic PDAC (Poplin et al., 2013).

Notably, a recent study showed that modulation of hENT1 expression levels altered the stiffness of PDAC and hENT1 knockdown induced epithelial to mesenchymal transition (EMT) in PDAC cells (Lee et al., 2014). However, several previous studies showed the key role of the EMT phenotype in acquired resistance of PDAC cells to gemcitabine (Shah et al., 2007; Wang et al., 2014). This is typically characterized by growth of pseudopodia, spindle-like shape, decreased E-cadherin expression and increased vimentin levels (i.e. increased cell stiffness and protection against compressive loads (Mendez et al., 2014)) in association with upregulation of Notch-2 (Arumugam et al., 2009; Wang et al., 2009).

In September 2013, the US Food and Drug Administration (FDA) approved the nanoparticle albumin-bound paclitaxel (nab-paclitaxel), in combination with gemcitabine, for first-line treatment of patients with metastatic PDAC. The higher tumor accumulation of nab-paclitaxel was hypothesized to be promoted by the presence of albumin-binding proteins, such as the secreted protein acidic and rich in cysteine (SPARC), which is overexpressed in stromal fibroblasts and down-regulated in tumor cells (Desai et al., 2009). In a phase I–II study, low SPARC expression correlated with significantly shorter survival; however, in the following phase III MPACT trial, SPARC failed both as a predictive biomarker and as a potential selection criterion for drug treatment with nab-paclitaxel (Hidalgo et al., 2015).

Analogously to the above gemcitabine-based regimens, the lack of predictive biomarkers is also a major clinical problem for FOLFIRINOX or FOLFIRINOX-modified treatments. A recent study showed that high levels of the enzyme carboxyl esterase-2 (CES2), which bioactivates the prodrug irinotecan to SN-38, was associated with longer OS and progression-free survival (PFS) in resectable and borderline-resectable patients treated with FOLFIRINOX in the neoadjuvant setting (Capello et al., 2015). However, these results, limited by the small number of patients (N = 22), and the expression data of resectable patients, might not be comparable to the data obtained with patients suffering from metastatic PDAC. Notably, a recent study on the mitogen-activated protein (MAP) kinase MAP4K5 showed that low expression levels of this protein correlated with the loss of E-cadherin and reduced CES2 expression in tissue specimens of 105 PDAC patients (Wang et al., 2016). This study suggests an important role for MAP4K5 in EMT, as well as in resistance to chemotherapy.

Several anti-apoptotic mechanisms can also decrease the efficacy of chemotherapeutic regimens (Neesse et al., 2013; Zhang et al., 2014). Recent studies showed that the Yes-associated protein (YAP) promotes cell survival by inhibiting pro-apoptotic signaling (Zhang et al., 2014). YAP overexpression in PDAC fostered tumor progression through the activation of the AKT cascade, which can counteract the effect of gemcitabine. These results suggested that YAP can act as a biomarker for predicting gemcitabine treatment response. Remarkably, YAP/TAZ have also been shown to act as sensors of the rigidity of the ECM by becoming activated on stiff substrates (15–40 kPa), thereby regulating mechanotransduction (Dupont et al., 2011).

2.1. Tumor microenvironment

PDAC exhibits a strong stromal reaction with only a minority of the tumor volume consisting of cancer cells (~10%). The heterogeneous tumor microenvironment (TME) is comprised of cellular and acellular stromal components, such as activated fibroblasts, myofibroblasts, pancreatic stellate cells (PSCs), immune cells, blood vessels, ECM, as well as soluble growth factors such as transforming growth factor-β (TGF-β), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and connective tissue growth factor (CTGF/CCN2) (Neesse et al., 2015). The TME is highly dynamic and continuously changing in composition, especially during the progression from pre-neoplastic lesions to invasive PDAC. The molecular mechanisms underlying the cross-talk between the TME and PDAC cells are extremely complex due to the heterogeneous nature of the PDAC stroma (Feig et al., 2012). Fibroblasts play a crucial role during the entire course of tumor development (Feig et al., 2012). They are transformed into cancer-associated fibroblasts (CAFs) through various growth factors and cytokines secreted by cancer cells. They are characterized by α-smooth muscle actin (α-SMA) expression, enhanced synthesis of collagens, ECM proteins and growth factors including TGF-β, EGF, PDGF (Feig et al., 2012).

A major source of PDAC CAFs is PSCs, which are resident mesenchymal cells of the pancreas that store lipid droplets and express fibroblast-activation protein α (FAP) (Apte et al., 2013). Activated PSCs are responsible for eliciting the stromal reaction in PDAC. During pancreatic injury, PSCs are activated, assume a myofibroblast-like phenotype and synthesize excessive amounts of ECM proteins, mostly collagen and fibronectin (FN), leading to fibrosis. They modulate the 3D collagen alignment to promote the migration of PDAC cells (Drifka et al., 2016) and interact with endothelial cells to stimulate angiogenesis (Apte and Wilson, 2012; Xu et al., 2014). Activated PSCs also express NADPH oxidase, a source of reactive oxygen species (ROS), which induces EMT and actin polymerization. Notably, a recent study identified two subtypes of PSC-derived CAFs, which may address the conflicting reports that have emerged in the field regarding CAF functions (Öhlund et al., 2017). CAFs with elevated expression of αSMA were indeed located immediately adjacent to neoplastic cells in mouse and human PDAC tissue, whereas co-cultures revealed a distinct subpopulation, located more distantly from neoplastic cells, which lacked elevated αSMA expression and instead, secreted IL-6 and inflammatory mediators. Therefore, the traditional view of the stroma as a uniformly pro-tumorigenic niche calls for reconsideration, as certain CAF subpopulations might have pro-tumorigenic features, whereas others might act as anti-tumorigenic factors, with possible implications for the development of therapeutic interventions.

Cumulative evidence support the key role of CAFs and myofibroblasts not only in shaping the soluble and solid stromal TME of PDAC, but also in the control of local immune suppression, thus promoting tumor progression (Watt and Kocher, 2013). FAP-expressing fibroblasts lead to immune suppression by CXCL12, the chemokine that signals via CXCR4. Depletion of FAP-positive CAFs permits immune control in various preclinical models of PDAC (Feig et al., 2013). These data further demonstrate that the immune-suppressive environment of PDAC is controlled by CAFs, which are therefore responsible for the failure of T-cell checkpoint antagonists.

2.1.1. Targeting stromal components

The dense and stiff ECM in PDAC compresses blood vessels, leading to reduced perfusion that ultimately impedes the delivery of chemotherapeutic drugs to tumor cells. Hence, this physical barrier highly contributes to the multidrug resistance phenotype to current chemotherapies.

The first preclinical study in genetically engineered mouse models (GEMMs) of PDAC that introduced the stromal depletion concept was conducted by Olive and colleagues (Olive et al., 2009). The authors demonstrated that pharmacological inhibition of the pro-stromal sonic hedgehog (Shh) signaling cascade by the Smoothened inhibitor saridegib (also known as IPI-926) led to a significant reduction of tumor stroma and increased perfusion and mean vessel density. Paralleled by these alterations, intratumoral gemcitabine delivery was elevated (i.e. increased by 60%) and therapeutic response and median survival increased significantly. Unfortunately, while inhibition of Shh can reduce the stromal component in PDAC, it might also promote a more vascularized and aggressive tumor, as demonstrated in a GEMM. These experimental evidence might explain the unsatisfactory results obtained with IPI-926, which has been studied in phase I and phase II trials. These trials were prematurely terminated because of the detrimental toxic side effect of the combination of IPI-926 and gemcitabine (Ko et al., 2016). Therefore, alternative strategies to target the stromal response in PDAC are critically needed.

Subsequent preclinical investigations introduced alternative approaches to successfully relieve vessel compression and improve drug delivery. For instance, hyaluronan (HA) is highly overexpressed in tumor cells and stromal cells and accumulates in PDAC (Kultti et al., 2014; Tammi et al., 2008). As a megadalton glycosaminoglycan, HA retains water due to its high colloid osmotic pressure and provides elasticity to connective tissue in healthy organs. An HA-rich, relatively immobile gel-fluid phase, induced vascular collapse and hypo-perfusion as a primary physical barrier increasing treatment resistance in PDAC (DuFort et al., 2016). In line with previous findings with a prostate cancer xenograft model (Thompson et al., 2010), preclinical trials were conducted in which degradation of HA was achieved by hyaluronidase PEGPH20 in pancreatic GEMM tumors (Jacobetz et al., 2012; Provenzano et al., 2012). A randomized phase II study of PEGPH20 plus nab-paclitaxel/gemcitabine in patients with untreated metastatic PDAC showed a statistically significant longer PFS, compared to nab-paclitaxel/gemcitabine (Hingorani et al., 2017). The largest improvement was observed in patients with high HA levels, as determined using a novel quantitative assay (VENTANA HA RxDx, Ventana Medical Systems, Inc.). Moreover, since unexpected, elevated risk of blood clots associated with PEGPH20 resulted in a temporary halt of this trial in 2014, the trial evaluated the use of enoxaparin (an anticoagulant that binds to antithrombin), which equalized the risk for thromboembolic events in the two arms. These data support HA as a potential predictive biomarker for patient selection of hyaluronidase PEGPH20, currently investigated in the ongoing global Phase III HALO-301 study (ClinicalTrials.gov: NCT02715804) with PFS and OS as co-primary endpoints.

Other investigators have shown that the angiotensin II receptor antagonist losartan decreases stromal collagen and secretion of HA in PDAC (Chauhan et al., 2013). Moreover, losartan treatment was accompanied by reduction of profibrotic signals such as TGF-β1, and CCN2/CTGF (Chauhan et al., 2013). The authors concluded that angiotensin receptor blockers (ARBs) reduce solid stress in tumors, resulting in increased vascular perfusion, oxygen flow and drug delivery. A currently ongoing phase II trial with FOLFIRINOX and losartan in patients with PDAC will determine whether or not this class of anti-hypertensive drugs may serve as an inexpensive therapeutic strategy to sensitize PDAC to first-line chemotherapy (NCT01821729).

Activation of the focal adhesion kinase (FAK) pathway is essential for promoting the desmoplastic TME of PDAC, and FAK inhibitors have demonstrated reasonable anti-tumor activity (Kanteti et al., 2016). Moreover, FAK plays a key role in regulating mechanical properties of cells required for cellular adhesion and motility (Mierke, 2013). The recent work by Jiang and colleagues has shown that FAK inhibition increases immune surveillance by overcoming the fibrotic and immunosuppressive PDAC TME and enhances tumor response to immunotherapy (Jiang et al., 2016). These results prompted a rapid clinical translation, resulting in a currently ongoing phase I clinical trial to test the anti-tumor efficacy of the FAK inhibitor defactinib in combination with pembrolizumab (anti-PD-1) and gemcitabine in advanced

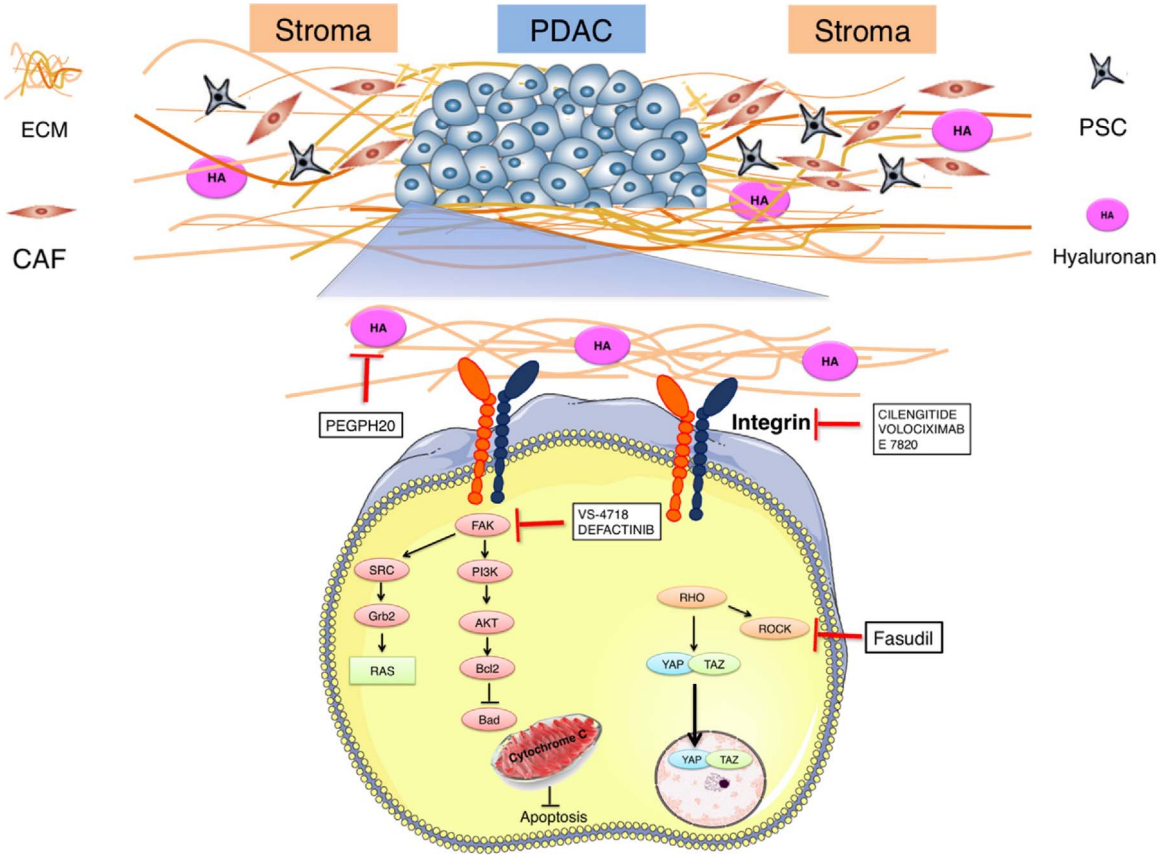

Fig. 1. Therapeutic targeting of stromal components, integrins and signaling pathways to overcome PDAC chemoresistance. The tumor microenvironment (TME) is composed of collagens, fibronectin, hyaluronan, an abundance of cancer-associated fibroblasts (CAFs), pancreatic stellate cells (PSCs), extracellular matrix (ECM) and other components. Integrin receptors have an affinity for collagen and fibronectin (FN). FN regulates cell proliferation through FAK-dependent recruitment of SH2-binding proteins such as Src and Grb2, which directly activate the Ras pathway. FN-mediated activation of FAK also produces pro-survival effect in cancer cells by activation of the PI3K/AKT/mTOR pathway. It results in upregulation of Bcl-2 through inhibition of Bad and blocked cytochrome c release from mitochondria leading to decreased apoptosis. Another pathway which drives cell growth and inhibits apoptosis involves Rho, together with Rho kinase ROCK in the regulation of yes-associated protein (YAP)/Tafazzin (TAZ) transcription activators by promoting the accumulation of these transcription activators in the nucleus. The specific therapeutic agents are listed in the boxes.

PDAC (NCT02546531). Another interesting approach to target FAK in PDAC, which combined the FAK inhibitor VS-4718 with gemcitabine and nab-paclitaxel (phase I, NCT02651727), was recently terminated by Verastem Inc., to de-prioritize VS-4718 development.

A seminal study by the Weaver group investigated the interplay between tumor genotype and fibrotic phenotype in PDAC progression (Laklai et al., 2016). Their findings implicated epithelial tension and matricellular fibrosis in the aggressiveness of mutant SMAD4 pancreatic tumors (disrupting TGF-β signaling) and highlighted STAT3, a transcription factor that regulates expression of pro-inflammatory genes, and functions as a key driver of this phenotype. This signaling arm acts via mechanoresponsive pathways in a feed-forward loop to amplify cellular responses that promote PDAC progression. The clinical relevance of these findings is supported by data from PDAC samples indicating that patients with shorter survival have elevated p-STAT3, p-MLC2, nuclear YAP levels and increased collagen bundling, which were also observed in patients with SMAD4 mutations. These remarkable findings should prompt further studies on the interactions between PDAC genotypes and mechanical properties of these tumors, as well as new therapeutic strategies targeting both tumor and stromal cells to reduce the tumor-promoting influence of the microenvironment.

Regarding alterations in TGF-β-signaling, all-*trans* retinoic acid (ATRA) hinders the capacity of PSCs to mechanically activate TGF-β (Sarper et al., 2016), thus preventing the fibrotic TME by PSC activation. Administration of ATRA in combination with gemcitabine in PDAC GEMMS resulted in a significant improvement of drug response (Guerra and Barbacid, 2013). Interestingly, ATRA reduced fibrosis and hypoxia, while enhancing tumor necrosis, and increased perfusion in PDAC (Carapuça et al., 2016). The ongoing phase I–II study STAR_PAC is repurposing ATRA as a stromal targeting agent along with gemcitabine and nab-paclitaxel for PDAC (Kocher et al., 2016).

More recently, Vennin and colleagues used intravital imaging to assess how transient priming of primary and secondary sites via Rho-associated protein kinase (ROCK) inhibition (i.e. inhibition of cellular tension/contractility) improves chemotherapy efficacy and retards the onset of metastasis in PDAC (Vennin et al., 2017). This study also demonstrated a graded response to priming in stratified patient-derived tumors, indicating that fine-tuned tissue manipulation before chemotherapy may offer opportunities in both primary and metastatic targeting of PDAC. A future phase I clinical trial will determine the safety of a transient priming regimen with the Rho kinase inhibitor Fasudil, prior to treatment with Gem/Abraxane in PDAC patients.

2.2. Integrin-dependent chemoresistance in PDAC

The fibrotic tissue present in PDAC tumors is characterized by an upregulated expression of the ECM to which both tumor and stroma (mostly activated PSCs) contribute (Feig et al., 2012). The PDAC ECM is composed mainly of collagens (type I and type IV) and fibronectin (FN), in addition to other molecules such as thrombospondin, periostin, tenascin C, vitronectin, versican, and biglycan (Feig et al., 2012).

Experimental evidence of reduced sensitivity to chemotherapeutic treatment when PDAC cells were attached to collagen (type I and type IV) and FN introduced the significant role of these ECM molecules in

drug resistance and tumor progression (Miyamoto et al., 2004).

Integrin receptors are transmembrane glycoproteins that are the major adhesion receptors for the ECM, consisting of one α and one β subunit. Both α1β1 and α2β1 bind type IV and type I collagens, though α1β1 has a higher affinity for type IV collagen and α2β1 for type I collagen. Whereas, both α5β1 and αvβ3 bind to FN, and both αvβ3 and αvβ5 bind to vitronectin. Integrin receptors known to bind to collagen (α2β1 and α1β1), to FN (α5β1 and αvβ3) and vitronectin (αvβ3 and αvβ5) are highly expressed by PDAC cells (Grzesiak and Bouvet, 2006). Notably, the malignant phenotype of PDAC cell lines was mediated by the α2β1 integrin-mediated adhesion to type I collagen (Grzesiak and Bouvet, 2006).

In addition to the physiological barrier that the ECM imposes, it also induces activation of intracellular signaling pathways important for growth and survival of cancer cells (Fig. 1). The pro-survival effect that FN has on cancer cells is primarily mediated by an FAK-dependent activation of the PI3K/AKT/mTOR pathway (Chen and Guan, 1994; Han et al., 2006). Activation of this pathway results in upregulation of Bcl-2 through inhibition of Bad, ultimately blocking cytochrome c release from mitochondria, which results in repression of apoptosis (Czabotar et al., 2014; Li et al., 1997). Immunohistochemical studies revealed that PDAC patients with the highest phospho-Akt levels had significantly shorter OS and PFS (Massihnia et al., 2017). Consistently, in PDAC cells characterized by high phospho-Akt expression, the combination of Akt inhibitors with gemcitabine increased apoptosis, associated with induction of caspase-3/6/8/9, PARP and BAD, and inhibition of Bcl-2 and NF-kB (Massihnia et al., 2017). Hence, these remarkable findings support the analysis of phospho-Akt expression as both a prognostic and a predictive biomarker, for the rational development of new combination therapies that target the Akt pathway in order to overcome gemcitabine-resistance. Furthermore, FN-mediated activation of FAK also triggers cell proliferation through the recruitment of SH2-binding proteins such as Src and Grb2, which directly activate the Ras pathway (Schlaepfer et al., 1994), as also reported, within an extensive description of the FN signaling in PDAC, by Topalovski and Brekken (Topalovski and Brekken, 2016). FN (and to a lesser extent collagen) also promotes PDAC cell survival through a modest increase in ROS. Mia PaCa-2, Panc-1, and Capan-1 tumor cells cultured on FN, stimulated NADPH-oxidase and 5-lipoxygenase-dependent ROS production (Edderkaoui et al., 2005). Consequently, these cells showed increased survival, which was reversed when ROS production was inhibited by treatment with antioxidants.

Moreover, α2β1 integrin protected PDAC cells from 5-FU-induced apoptosis by upregulating the anti-apoptotic Bcl-2 family member Mcl-1 (Armstrong et al., 2004). Apart from directly regulating apoptotic signaling, α2β1 integrin promoted the resistance of PDAC cells to gemcitabine by increasing MT-MMP-1-mediated ERK phosphorylation and increased expression of the chromatin remodeling protein high mobility group A2 and of histone acetyl-transferases (Dangi-Garimella et al., 2011; Dangi-Garimella et al., 2013).

2.2.1. *Targeting integrins*

Abundant collagen deposition is a hallmark of the desmoplastic reaction in both primary and metastatic lesions of PDAC (Whatcott et al., 2015). Through integrin signaling, deposited collagen increases tumor cell proliferation, survival and chemoresistance, possibly contributing to further establishment of metastatic lesions. These biological properties of integrins as well as their ability to crosstalk with growth factor receptors has rendered them attractive druggable targets, and several preclinical studies showed that integrin antagonists inhibit tumor growth by affecting both tumor cells and tumor-associated host cells (Desgrosellier and Cheresh, 2010). However, further clinical studies will have to elucidate how effective these agents are as cancer therapeutics.

Cilengitide (EMD 121974) is a low-molecular weight cyclic peptide inhibitor of αvβ3 and αvβ5. These integrins are surface receptors which are not expressed in normal tissues but are upregulated on the surface of endothelial cells in blood vessels undergoing angiogenesis (Brooks et al., 1994). Remarkably, both αvβ3 and αvβ5 are expressed on PDAC cells (Grzesiak and Bouvet, 2006). In pre-clinical studies, cilengitide inhibited tumor-mediated angiogenesis and the growth of human tumor xenografts (Brooks et al., 1994; MacDonald et al., 2001). *In vitro* studies have also shown that cilengitide not only inhibits angiogenesis but also displays direct cytotoxic activity in αvβ3- and αvβ5-expressing tumor cell lines (Taga et al., 2002). However, a phase II trial showed that, in comparison to gemcitabine, the combination of cilengitide and gemcitabine had no significant benefits for patients with advanced unresectable PDAC (Friess et al., 2006). This failure might be attributed both to the very short half-life of this compound *in vivo* as well as to the relative minor role of metastasis formation (Giovannetti et al., 2017), which is considered the main target of this drug, in patients who have already advanced/metastatic PDAC.

Volociximab is an anti-α5β1 integrin monoclonal antibody that displayed inhibition of tumor growth in various animal tumor models (Ng et al., 2010). Phase I clinical trials showed that volociximab was well tolerated by patients and entered phase II clinical trials for the treatment of metastatic PDAC (Evans et al., 2008). Despite the promising preliminary effects of volociximab, particularly when paired with standard chemotherapy such as gemcitabine, the overall results have been modest.

E7820 is an aromatic sulfonamide that inhibits integrin α2 mRNA expression. In pre-clinical studies, treatment with E7820 inhibited tumor growth and tumor-induced angiogenesis in mouse xenografts derived from different solid tumors, with complete suppression of growth of human PDAC models (Semba et al., 2004). An ongoing phase I trial demonstrated that the tolerability of E7820 was acceptable and no significant safety concerns were identified, warranting further development of this drug in gastrointestinal malignancies (NCT01773421) (Mita et al., 2011).

The safety and tolerability of integrin inhibitors with diverse molecular structures have been largely confirmed by several studies. However, the clinical trials with cilengitide, voloxicimab and E7820 reported only prolonged stable disease as best tumor response without survival benefits compared to single agent approaches for the treatment of PDAC. These disappointing clinical results clearly emphasize the need to identify how key PDAC tumors and ECM factors might influence the susceptibility to these inhibitors. This is particularly true since integrins recognize specific motifs in the ECM. Importantly, integrins form the mechanical coupling between the ECM and the cytoskeletal machinery, and are therefore key in both rigidity sensing of the ECM by the cell, and the application of traction forces on the microenvironment. However, the ECM mesh size, nanotopography, the thickness and mechanics of the constituent fibers influence cell behavior in complex ways (Jansen et al., 2015). A key challenge for future research is therefore to design physiologically relevant assays that can unravel these effects.

3. Tools to investigate PDAC mechanopharmacology

Mechanopharmacology requires the combination of tools and concepts established in biophysics, engineering and biology. Over the past two decades, the advances in high-resolution microscopy and in mechanobiology tools paved the way to investigate with unprecedented resolution capacity, the role of mechanics in health and disease (Eisenstein, 2017). We will present below representative powerful techniques that are capable of monitoring how cells apply forces, alter their environment ('inside-out signaling') and, *vice versa*, how cells probe the mechanical properties of their microenvironment and transduce this information together with information from other signals such as growth factors into a concerted response ('outside-in coupling'). Comprehensive reports have been published by Chen and colleagues (Eyckmans et al., 2011; Polacheck and Chen, 2016). However, Fig. 2

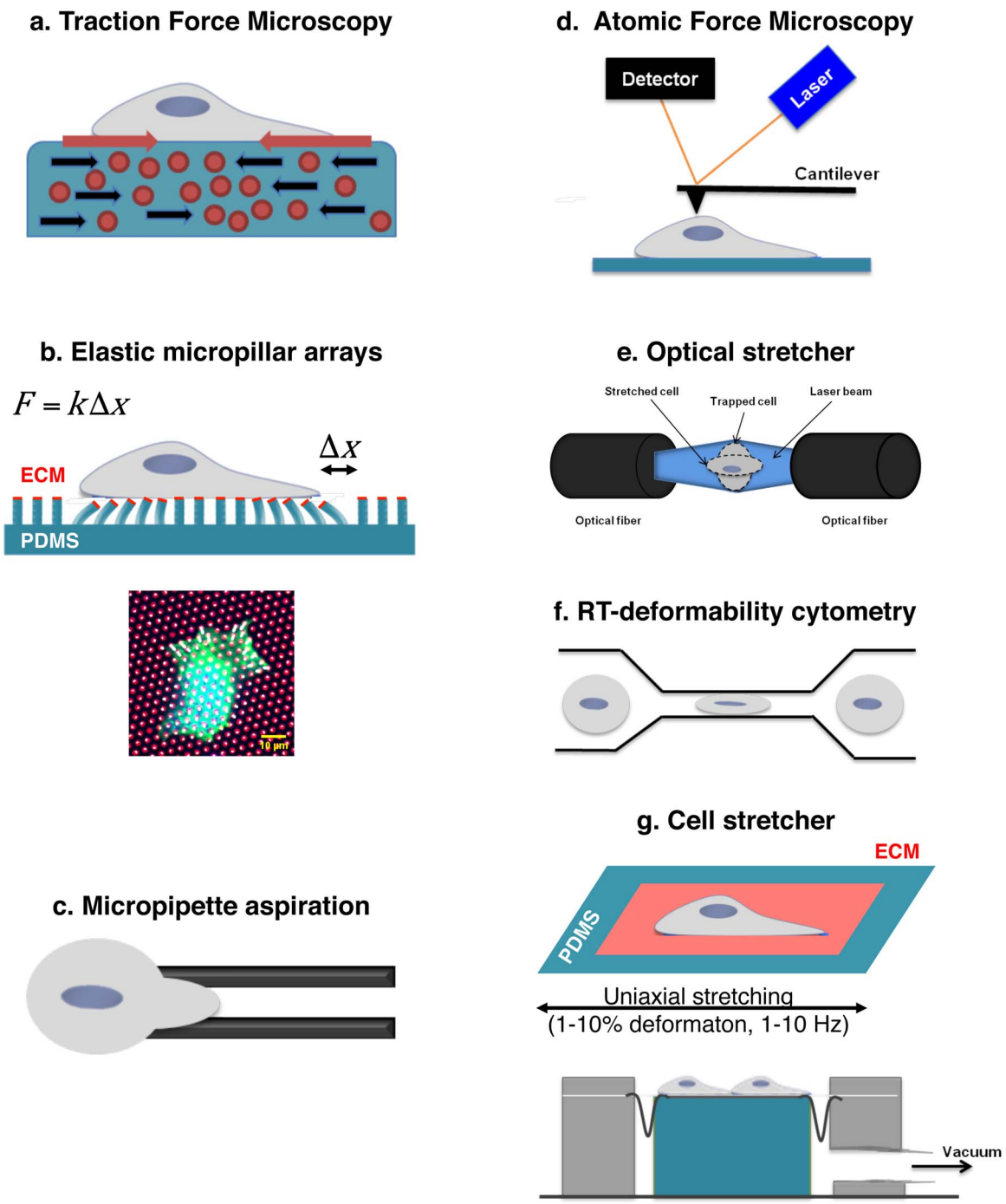

Fig. 2. Experimental tools in mechanobiology to study chemoresistance in PDAC. a–b. Traction force microscopy and elastic micropillar arrays are tools to investigate the inside-out signaling by measuring force generation by adherent cells on 2D substrates of controlled stiffness. **c–f.** Micropipette aspiration, atomic force microscopy, optical stretcher and RT-deformability allow us to study the viscoelastic properties of cells and tissues. **g.** The cell stretcher device (uniaxial, top or equi-biaxial, bottom) permits to elucidate the outside-in signaling by active application of mechanical stress with tunable amplitude and frequency. All tools are combined with high- (super-) resolution microscopy techniques to increase the temporal and spatial resolution.

exemplifies the most important tools, with specific application to pre-clinical models of PDAC.

3.1. Traction force microscopy on elastic substrates

In 1980, Harris and colleagues were the first to demonstrate deformations generated by fibroblasts on soft silicone rubber substrates (Harris et al., 1980). Further developments over the last 30 years led to the development of traction force microscopy (TFM) technique that allows to extract the traction forces generated by cells with improved resolution, accuracy, and reproducibility by combining high-resolution optical imaging and extensive computational analyses (Plotnikov et al., 2014). TFM is a method to map and determine traction forces exerted by adherent cells on continuous, linearly elastic, usually two-dimensional hydrogels (e.g. polyacrylamide) with fluorescent tracer particles embedded within the gel (Fig. 2a). The hydrogel stiffness is tuned by adjusting the acrylamide monomer and cross-linker content in polyacrylamide gels (Denisin and Pruitt, 2016). The substrate is deformed by the cells and the displacement is measured by tracking the tracer particles. The resulting gel displacements can be converted into traction

forces by using the material constitutive relations and solving the elastic problem subject to the appropriate boundary condition (Style et al., 2014; Sabass et al., 2008; Trepat et al., 2009). The covalent cross-linking of the substrate with specific ECM proteins (e.g. fibronectin) allows to selectively activate distinct classes of adhesion receptors. The introduction of high density of two color fluorescent microspheres allowed to measure traction forces within individual focal adhesions (with up to 50 markers per focal adhesion) on polyacrylamide gels down to 1 μm resolution (Sabass et al., 2008; Plotnikov Sergey et al., 2017). More recently, the combination of stimulated emission depletion (STED) microscopy and TFM permitted to detect forces at the nanoscale (Colin-York et al., 2016). Notably, other groups are expanding the technique to three dimensions in order to quantify the cell-generated forces in highly nonlinear 3D biopolymer (e.g. collagen gels, fibril gels and Matrigel) networks (Steinwachs et al., 2016).

3.2. Elastic micropillar arrays

Elastic micropillars are powerful tools to quantify the forces that adhering cells exert on their substrates ('inside-out signaling') (Fig. 2b). One widely used design, developed by the bioengineer Christopher Chen and his colleagues (Tan et al., 2003), consists of an ordered (e.g. hexagonal patterned) array of evenly spaced and flexible pillars made of poly dimethyl-siloxane (PDMS). A precise control of the physical properties of the environment is achieved by tuning the pillar geometry (e.g. diameter, spacing, length). The arrays can be produced using replica-molding from a silicon wafer into which the negative of the structure (i.e. the pattern of micrometer-wide holes of varying depth) is etched by e.g. deep reactive-ion etching. The tops of the micropillars are coated with the ECM molecule of interest, for instance FN, using micro-contact printing, whereas the rest of the array is coated with anti-adsorption materials. This ensures that once plated, cells solely adhere to the pillar tops. Fluorescent labeling of ECM molecules (for example, in a ratio of 1:5 to unlabeled components), allows localization of the pillar-centroids by high-resolution (i.e. high-NA objective) fluorescence microscopy, down to ~30 nm resolution at an imaging rate of 100 images/sec (van Hoorn et al., 2014). The force-deflection relation of pillars is well approximated by Hooke's law and can be precisely calibrated when combining finite element methods and electron microscopy measurements of the micropillars. Distinct pillar lengths correspond to distinct spring-constants together with the precision to which deflections of ~30 nm can be resolved, corresponding to a force accuracy of ~500 pN (van Hoorn et al., 2014). Remarkably, elastic micropillars can be combined with super-resolution methods (van Hoorn et al., 2014) to investigate the events that produce cellular forces at the single molecule level.

3.3. Rheometry on individual cells

The rheological properties of cells and tissues are sensitive indicators of physiological and pathological changes (Guo et al., 2014; Rigato et al., 2017). Micropipette aspiration is regarded as a pioneering technique for single cell elasticity measurement (yielding values of the Young's modulus ranging from 100 Pa to 500 Pa) by the observation of cell deformation upon pressure suction (Fig. 2c) (Hochmuth, 2016). A conventional micropipette aspiration system generally consists of a glass capillary micropipette for the suction and simultaneous optical access to observe the cell deformation. Despite its simplicity, micropipette aspiration has been applied to a variety of experimental systems that span different length scales to study cell mechanics, nanoscale molecular mechanisms in single cells, bleb growth, and nucleus dynamics.

Atomic force microscopy (AFM) is a powerful method to study biophysical properties in a broad length scale, commonly used in combination with high-resolution microscopes for simultaneous imaging of the processes occurring in the sample (Haase and Pelling, 2015; Sen et al., 2016). The surface under investigation is mechanically indented by a cantilever with a very sharp tip (~10 nm), whose deflections (with respect to the equilibrium position) are detected by a photodiode (Fig. 2d). The use of spherical tips allows AFM to measure the elasticity of living cells yielding values of the Young's modulus ranging from 100 Pa to 100 kPa, avoiding artifacts introduced by extraneous cantilever-cell contact (Harris and Charras, 2011).

In 2001, Guck and colleagues developed the optical stretcher (OS), a laser tool to manipulate single cells (Fig. 2e) (Guck et al., 2001). The basic principle is that the surface forces acting on a dielectric object placed between two opposed, non-focused laser beams, lead to a stretching of the object along the axis of the beams. The method had the sensitivity necessary to distinguish even between different individual cytoskeletal phenotypes in fibroblasts. More recently, Guck and colleagues developed a new method, real-time deformability cytometry (RT-DC), to probe cell stiffness at high throughput (> 100 cells/sec) by exposing cells to a shear flow in a microfluidic channel, allowing for mechanical phenotyping based on single-cell deformability (Fig. 2f) (Otto et al., 2015; Mietke et al., 2015). RT-DC is capable of distinguishing between specific cell-cycle phases, track stem cell differentiation into distinct lineages and identify cell populations in whole blood by their mechanical fingerprints.

3.4. Support-based cell stretcher

The cell stretcher is a device to investigate the ability of adherent cells to sense and respond to mechanical stimuli ('outside-in coupling'). The uniaxial system can be based on a variable stroke cam-lever-tappet mechanism, which allows the delivery of cyclic stimuli with tunable frequency (up to 10 Hz), duration and displacement (deformation between 1% and 20%) (Fig. 2g, top) (Balcioglu et al., 2015; Kamble et al., 2016; Seriani et al., 2016). The cells are plated on an *ad hoc* PDMS membrane, coated with ECM molecules (e.g. FN), which is then loaded on the clamps of the cell-stretcher. As for the micropillar arrays, the cell stretcher device can be optically accessed and the response of cells to cyclic stimuli (e.g. cytoskeleton reorganization, pathways activation) is monitored by high-resolution imaging (Balcioglu et al., 2015). Other designs allow equi-biaxial stretching through the cell substrate deformation by applying vacuum underneath the flexible-bottomed well (Fig. 2g, bottom) (Hung and Williams, 1994).

3.5. Mechanical and physical aspects of multi-cellular 3D systems

It is now well accepted that organotypic 3D systems greatly serve as therapy test platforms to predict clinical efficacies (Friedrich et al., 2009). Therefore, a physical characterization of multi-cellular 3D systems is also required to be successful in the mechanopharmacology approach (Gilbert and Weaver, 2017). In the last decade, several tools have been developed to apply and measure mechanical stresses beyond the single cell scale (Warren et al., 2016; Eyckmans and Chen, 2017; Shao et al., 2015). Guevorkian and colleagues estimated the surface tension, viscosity, and elastic modulus of spherical cellular aggregates (spheroids), using a micropipette aspiration-based technique (Guevorkian et al., 2010). Novel methods for molding spheroids, deforming them and measuring elastic properties through magnetic nanoparticles have been recently designed to study the effect of an applied mechanical stress (Mazuel et al., 2015; Montel et al., 2011). On the contrary, biocompatible, magnetically responsive ferrofluid microdroplets have been introduced as local mechanical actuators to measure cell-generated mechanical stresses *in vitro* and *in vivo* (Lucio et al., 2015; Serwane et al., 2017).

4. Concluding remarks

In past two decades, concentrated efforts to elucidate the genetic and molecular mechanisms underlying PDAC initiation and progression

modestly contributed to the reduction of the tumor burden in this lethal disease. The yet unsatisfactory results of available treatments targeting the stromal cellular/non-cellular components, stem from the complexity of intrinsic and acquired chemoresistance modalities in PDAC, fostered by the mutational landscape and genomic instability as well as the mechanical tumor-stroma interaction. The mechanopharmacology approach, aiming at unraveling the bidirectional interplay between drug action/resistance and mechanics, should be successful at identifying new drugs and drug targets towards the eradication of PDAC, as well as predictive/prognostic biomarkers that are yet representing a clinical unmet problem. Furthermore, mechanopharmacology should provide a better understanding of the failures of current treatments, e.g. by clarifying the importance and contribution of each integrin to PDAC chemoresistance.

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References

Apte, M.V., Wilson, J.S., 2012. Dangerous liaisons: pancreatic stellate cells and pancreatic cancer cells. J. Gastroenterol. Hepatol. 27 (s2), 69–74.
Apte, M.V., Wilson, J.S., Lugea, A., Pandol, S.J., 2013. A starring role for stellate cells in the pancreatic cancer microenvironment. Gastroenterology 144 (6), 1210–1219.
Armstrong, T., et al., 2004. Type I collagen promotes the malignant phenotype of pancreatic ductal adenocarcinoma. Clin. Cancer Res. 10 (21), 7427–7437.
Arumugam, T., et al., 2009. Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. Cancer Res. 69 (14), 5820–5828.
Bailey, P., et al., 2016. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 531 (7592), 47–52.
Balcioglu, H.E., van Hoorn, H., Donato, D.M., Schmidt, T., Danen, E.H., 2015. The integrin expression profile modulates orientation and dynamics of force transmission at cell–matrix adhesions. J. Cell Sci. 128 (7), 1316–1326.
Bramhall, S., Rosemurgy, A., Brown, P., Bowry, C., Buckels, J., 2001. Marimastat as first-line therapy for patients with unresectable pancreatic cancer: a randomized trial. J. Clin. Oncol. 19 (15), 3447–3455.
Bramhall, S., et al., 2002. A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer. Br. J. Cancer 87 (2), 161–167.
Brooks, P.C., Clark, R.A., Cheresh, D.A., 1994. Requirement of vascular integrin alpha-v beta-3 for angiogenesis. Science 264 (5158), 569–572.
Capello, M., et al., 2015. Carboxylesterase 2 as a determinant of response to irinotecan and neoadjuvant FOLFIRINOX therapy in pancreatic ductal adenocarcinoma. J. Natl. Cancer Inst. 107 (8) djv132.
Carapuça, E.F., et al., 2016. Anti-stromal treatment together with chemotherapy targets multiple signalling pathways in pancreatic adenocarcinoma. J. Pathol. 239 (3), 286–296.
Chauhan, V.P., et al., 2013. Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumour blood vessels. Nat. Commun. 4, 2516. http://dx.doi.org/10.1038/ncomms3516.
Chen, H.-C., Guan, J.-L., 1994. Association of focal adhesion kinase with its potential substrate phosphatidylinositol 3-kinase. Proc. Natl. Acad. Sci. 91 (21), 10148–10152.
Chu, G.C., Kimmelman, A.C., Hezel, A.F., DePinho, R.A., 2007. Stromal biology of pancreatic cancer. J. Cell. Biochem. 101 (4), 887–907.
Colin-York, H., et al., 2016. Super-resolved traction force microscopy (STFM). Nano Lett. 16 (4), 2633–2638.
Conroy, T., et al., 2011. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. New Engl. J. Med. 364 (19), 1817–1825.
Czabotar, P.E., Lessene, G., Strasser, A., Adams, J.M., 2014. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat. Rev. Mol. Cell Biol. 15 (1), 49–63.
Dangi-Garimella, S., et al., 2011. Three-dimensional collagen I promotes gemcitabine resistance in pancreatic cancer through MT1-MMP–mediated expression of HMGA2. Cancer Res. 71 (3), 1019–1028.
Dangi-Garimella, S., Sahai, V., Ebine, K., Kumar, K., Munshi, H.G., 2013. Three-dimensional collagen I promotes gemcitabine resistance in vitro in pancreatic cancer cells through HMGA2-dependent histone acetyltransferase expression. PLoS One 8 (5), e64566.
Denisin, A.K., Pruitt, B.L., 2016. Tuning the range of polyacrylamide gel stiffness for mechanobiology applications. ACS Appl. Mater. Interfaces 8 (34), 21893–21902.
Desai, N., Trieu, V., Damascelli, B., Soon-Shiong, P., 2009. SPARC expression correlates with tumor response to albumin-bound paclitaxel in head and neck cancer patients. Transl. Oncol. 2 (2), 59–64.
Desgrosellier, J.S., Cheresh, D.A., 2010. Integrins in cancer: biological implications and therapeutic opportunities. Nat. Rev. Cancer 10 (1), 9–22.
Drifka, C.R., et al., 2016. Human pancreatic stellate cells modulate 3D collagen alignment to promote the migration of pancreatic ductal adenocarcinoma cells. Biomed. Microdevices 18 (6), 105.
DuFort, C.C., DelGiorno, K.E., Hingorani, S.R., 2016. Mounting pressure in the microenvironment: fluids, solids, and cells in pancreatic ductal adenocarcinoma. Gastroenterology 1557, e1542.
Dupont, S., et al., 2011. Role of YAP/TAZ in mechanotransduction. Nature 474 (7350), 179–183.
Edderkaoui, M., et al., 2005. Extracellular matrix stimulates reactive oxygen species production and increases pancreatic cancer cell survival through 5-lipoxygenase and NADPH oxidase. Am. J. Physiol. Gastrointest. Liver Physiol. 289 (6), G1137–G1147.
Eisenstein, M., 2017. Mechanobiology: a measure of molecular muscle. Nature 544 (7649), 255–257.
Evans, T., et al., 2008. Interim results from a phase II study of volociximab in combination with gemcitabine (GEM) in patients (pts) with metastatic pancreatic cancer (MPC). Gastrointestinal Cancers Symposium Abstract No: 142.
Eyckmans, J., Chen, C.S., 2017. 3D culture models of tissues under tension. J. Cell Sci. 130 (1), 63–70.
Eyckmans, J., Boudou, T., Yu, X., Chen, C.S., 2011. A hitchhiker's guide to mechanobiology. Dev. Cell 21 (1), 35–47.
Farrell, J.J., et al., 2009. Human equilibrative nucleoside transporter 1 levels predict response to gemcitabine in patients with pancreatic cancer. Gastroenterology 136 (1), 187–195.
Feig, C., et al., 2012. The pancreas cancer microenvironment. Clin Cancer Res. 18 (16), 4266–4276. http://dx.doi.org/10.1158/1078-0432.CCR-11-3114.
Feig, C., et al., 2013. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti–PD-L1 immunotherapy in pancreatic cancer. Proc. Natl. Acad. Sci. 110 (50), 20212–20217.
Friedrich, J., Seidel, C., Ebner, R., Kunz-Schughart, L.A., 2009. Spheroid-based drug screen: considerations and practical approach. Nat. Protoc. 4 (3), 309–324.
Friess, H., et al., 2006. A randomized multi-center phase II trial of the angiogenesis inhibitor Cilengitide (EMD 121974 and gemcitabine compared with gemcitabine alone in advanced unresectable pancreatic cancer. BMC Cancer 6 (1), 285.
Gilbert, P.M., Weaver, V.M., 2017. Cellular adaptation to biomechanical stress across length scales in tissue homeostasis and disease. Semin. Cell Dev. Biol. 67, 141–152.
Giovannetti, E., et al., 2017. Never let it go: stopping key mechanisms underlying metastasis to fight pancreatic cancer. Semin. Cancer Biol. 44, 43–59.
Gonen, N., Assaraf, Y.G., 2016. Antifolates in cancer therapy: structure, activity and mechanisms of drug resistance. Drug Resist. Updat. 15 (4), 183–210.
Gore, J., Korc, M., 2014. Pancreatic cancer stroma: friend or foe? Cancer Cell 25 (6), 711–712.
Greenhalf, W., et al., 2014. Pancreatic cancer hENT1 expression and survival from gemcitabine in patients from the ESPAC-3 trial. JNCI: J. Natl. Cancer Inst. 106 (1).
Grzesiak, J., Bouvet, M., 2006. The α2β1 integrin mediates the malignant phenotype on type I collagen in pancreatic cancer cell lines. Br. J. Cancer 94 (9), 1311–1319.
Guck, J., et al., 2001. The optical stretcher: a novel laser tool to micromanipulate cells. Biophys. J . 81 (2), 767–784.
Guerra, C., Barbacid, M., 2013. Genetically engineered mouse models of pancreatic adenocarcinoma. Mol. Oncol. 7 (2), 232–247.
Guevorkian, K., Colbert, M.-J., Durth, M., Dufour, S., Brochard-Wyart, F., 2010. Aspiration of biological viscoelastic drops. Phys. Rev. Lett. 104 (21), 218101.
Guo, M., et al., 2014. Probing the stochastic, motor-driven properties of the cytoplasm using force spectrum microscopy. Cell 158 (4), 822–832.
Haase, K., Pelling, A.E., 2015. Investigating cell mechanics with atomic force microscopy. J. R. Soc. Interface 12 (104), 20140970.
Han, S., Khuri, F.R., Roman, J., 2006. Fibronectin stimulates non–small cell lung carcinoma cell growth through activation of Akt/mammalian target of rapamycin/S6 kinase and inactivation of LKB1/AMP-activated protein kinase signal pathways. Cancer Res. 66 (1), 315–323.
Harris, A.R., Charras, G., 2011. Experimental validation of atomic force microscopy-based cell elasticity measurements. Nanotechnology 22 (34), 345102.
Harris, A.K., Wild, P., Stopak, D., 1980. Silicone rubber substrata: a new wrinkle in the study of cell locomotion. Science 208 (4440), 177–179.
Hidalgo, M., et al., 2015. SPARC expression did not predict efficacy of nab-paclitaxel plus gemcitabine or gemcitabine glone for metastatic pancreatic cancer in an exploratory analysis of the phase III MPACT trial. Clin. Cancer Res. 21 (21), 4811–4818.
Hingorani, S.R., et al., 2017. Randomized phase II study of PEGPH20 plus nab-paclitaxel/gemcitabine (PAG) vs AG in patients (Pts) with untreated, metastatic pancreatic ductal adenocarcinoma (mPDA). J. Clin. Oncol. 35 4008–4008.
Hochmuth, R.M., 2016. Micropipette aspiration of living cells. J. Biomech. 33 (1), 15–22.
Hung, C., Williams, J., 1994. A method for inducing equi-biaxial and uniform strains in elastomeric membranes used as cell substrates. J. Biomech. 27 (2), 227–232.
Jacobetz, M.A., et al., 2012. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. Gut:gutjnl 2012–302529.
Jansen, K.A., et al., 2015. A guide to mechanobiology: where biology and physics meet. Biochim. Biophys. Acta. 1853 (11 Pt B), 3043–3052.
Jiang, H., et al., 2016. Targeting focal adhesion kinase renders pancreatic cancers responsive to checkpoint immunotherapy. Nat. Med. 22 (8), 851–860.
Kamble, H., Barton, M.J., Jun, M., Park, S., Nguyen, N.-T., 2016. Cell stretching devices as research tools: engineering and biological considerations. Lab Chip 16 (17), 3193–3203.
Kanteti, R., Batra, S.K., Lennon, F.E., Salgia, R., 2016. FAK and paxillin, two potential targets in pancreatic cancer. Oncotarget 7 (21), 31586.

Kleeff, J., et al., 2016. Pancreatic cancer. Nat. Rev. Dis. Primers 21 (2), 16022. http://dx.doi.org/10.1038/nrdp.2016.22.
Ko, A.H., et al., 2016. A phase I study of FOLFIRINOX plus IPI-926, a hedgehog pathway inhibitor, for advanced pancreatic adenocarcinoma. Pancreas 45 (3), 370–375.
Kocher, H., et al., 2016. STAR_PAC: A Phase 1 B study repurposing ATRA as stromal targeting agent along with gemcitabine and nab¬ Paclitaxel for pancreatic cancer. Pancreatology 16 (3), S4.
Krishnan, R., Park, J.-A., Seow, C.Y., Lee, P.V., Stewart, A.G., 2016. Cellular biomechanics in drug screening and evaluation: mechanopharmacology. Trends Pharmacol. Sci. 37 (2), 87–100.
Kultti, A., et al., 2014. Accumulation of extracellular hyaluronan by hyaluronan synthase 3 promotes tumor growth and modulates the pancreatic cancer microenvironment. BioMed Res. Int. 2014.
Laklai, H., et al., 2016. Genotype tunes pancreatic ductal adenocarcinoma tissue tension to induce matricellular fibrosis and tumor progression. Nat. Med. 22, 497–505.
Lee, Y., et al., 2014. Human equilibrative nucleoside transporter-1 knockdown tunes cellular mechanics through epithelial-mesenchymal transition in pancreatic cancer cells. PLoS One 9 (10), e107973.
Li, P., et al., 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91 (4), 479–489.
Li, W., et al., 2017. Overcoming ABC transporter-mediated multidrug resistance: molecular mechanisms and novel therapeutic drug strategies. Drug Resist. Updat. 27, 14–29.
Lucio, A.A., Ingber, D.E., Campas, O., 2015. Generation of biocompatible droplets for in vivo and in vitro measurement of cell-generated mechanical stresses. Methods Cell Biol. 125, 373–390.
MacDonald, T.J., et al., 2001. Preferential susceptibility of brain tumors to the anti-angiogenic effects of an αv integrin antagonist. Neurosurgery 48 (1), 151–157.
Massihnia, D., et al., 2017. Phospho-Akt overexpression is prognostic and can be used to tailor the synergistic interaction of Akt inhibitors with gemcitabine in pancreatic cancer. J. Hematol. Oncol. 10 (1), 9.
Mazuel, F., et al., 2015. Magnetic flattening of stem-cell spheroids indicates a size-dependent elastocapillary transition. Phys. Rev. Lett. 114 (9) 098105.
Mendez, M., Restle, D., Janmey, P., 2014. Vimentin enhances cell elastic behavior and protects against compressive stress. Biophys. J . 107 (2), 314–323.
Mierke, C.T., 2013. The role of focal adhesion kinase in the regulation of cellular mechanical properties. Phys. Biol. 10 (6), 065005.
Mietke, A., et al., 2015. Extracting cell stiffness from real-time deformability cytometry: theory and experiment. Biophys. J . 109 (10), 2023–2036.
Mita, M., et al., 2011. Phase I study of E7820, an oral inhibitor of integrin α-2 expression with antiangiogenic properties, in patients with advanced malignancies. Clin. Cancer Res. 17 (1), 193–200.
Miyamoto, H., et al., 2004. Tumor-stroma interaction of human pancreatic cancer: acquired resistance to anticancer drugs and proliferation regulation is dependent on extracellular matrix proteins. Pancreas 28 (1), 38–44.
Montel, F., et al., 2011. Stress clamp experiments on multicellular tumor spheroids. Phys. Rev. Lett. 107 (18) 188102.
Moore, M., et al., 2003. Comparison of gemcitabine versus the matrix metalloproteinase inhibitor BAY 12-9566 in patients with advanced or metastatic adenocarcinoma of the pancreas: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. J. Clin. Oncol. 21 (17), 3296–3302.
Neesse, A., et al., 2013. CTGF antagonism with mAb FG-3019 enhances chemotherapy response without increasing drug delivery in murine ductal pancreas cancer. Proc. Natl. Acad. Sci. 110 (30), 12325–12330.
Neesse, A., Krug, S., Gress, T.M., Tuveson, D.A., Michl, P., 2014. Emerging concepts in pancreatic cancer medicine: targeting the tumor stroma. OncoTargets Therapy 7, 33.
Neesse, A., Algül, H., Tuveson, D.A., Gress, T.M., 2015. Stromal biology and therapy in pancreatic cancer: a changing paradigm. Gut:gutjnl 2015–309304.
Ng, C.M., Bai, S., Takimoto, C., Tang, M., Tolcher, A., 2010. Mechanism-based receptor-binding model to describe the pharmacokinetic and pharmacodynamic of an anti-α5β1 integrin monoclonal antibody (volociximab) in cancer patients. Cancer Chemother. Pharmacol. 65 (2), 207.
Nordh, S., Ansari, D., Andersson, R., 2014. hENT1 expression is predictive of gemcitabine outcome in pancreatic cancer: a systematic review. World J. Gastroenterol. 20 (26), 8482–8490.
Öhlund, D., et al., 2017. Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer. J. Exp. Med. 214 (3), 579–596.
Olive, K.P., et al., 2009. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 324 (5933), 1457–1461.
Otto, O., et al., 2015. Real-time deformability cytometry: on-the-fly cell mechanical phenotyping. Nat. Meth. 12 (3), 199–202.
Paulson, A.S., Cao, H.S.T., Tempero, M.A., Lowy, A.M., 2013. Therapeutic advances in pancreatic cancer. Gastroenterology 144 (6), 1316–1326.
Plotnikov Sergey, V., Pasapera Ana, M., Sabass, B., Waterman Clare, M., 2017. Force fluctuations within focal adhesions mediate ECM-rigidity sensing to guide directed cell migration. Cell 151 (7), 1513–1527.
Plotnikov, S.V., Sabass, B., Schwarz, U.S., Waterman, C.M., 2014. High-resolution traction force microscopy. Methods Cell Biol. 123, 367.
Polacheck, W.J., Chen, C.S., 2016. Measuring cell-generated forces: a guide to the available tools. Nat. Methods 13 (5), 415–423.
Poplin, E., et al., 2013. Randomized, multicenter, phase II study of CO-101 versus gemcitabine in patients with metastatic pancreatic ductal adenocarcinoma: including a prospective evaluation of the role of hENT1 in gemcitabine or CO-101 sensitivity. J. Clin. Oncol. 31 (35), 4453–4461.
Provenzano, P.P., et al., 2012. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer Cell 21 (3), 418–429.
Rahib, L., et al., 2014. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res. 74 (11), 2913–2921.
Rigato, A., Miyagi, A., Scheuring, S., Rico, F., 2017. High-frequency microrheology reveals cytoskeleton dynamics in living cells. Nat. Phys. http://dx.doi.org/10.1038/nphys4104.
Sabass, B., Gardel, M.L., Waterman, C.M., Schwarz, U.S., 2008. High resolution traction force microscopy based on experimental and computational advances. Biophys. J . 94 (1), 207–220.
Sarper, M., Cortes, E., Lieberthal, T.J., del Río Hernández, A., 2016. ATRA modulates mechanical activation of TGF-β by pancreatic stellate cells. Sci. Rep. 6, 27639. http://dx.doi.org/10.1038/srep27639.
Schlaepfer, D.D., Hanks, S.K., Hunter, T., van der Geer, P., 1994. Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. Nature 372 (6508), 786.
Semba, T., et al., 2004. An angiogenesis inhibitor E7820 shows broad-spectrum tumor growth inhibition in a Xenograft model. Clin. Cancer Res. 10 (4), 1430–1438.
Sen, S., Subramanian, S., Discher, D.E., 2016. Indentation and adhesive probing of a cell membrane with AFM: theoretical model and experiments. Biophys. J . 89 (5), 3203–3213.
Seriani, S., et al., 2016. The cell-stretcher: a novel device for the mechanical stimulation of cell populations. Rev. Sci. Instrum. 87 (8), 084301.
Serwane, F., et al., 2017. In vivo quantification of spatially varying mechanical properties in developing tissues. Nat. Methods 14 (2), 181–186.
Shah, A.N., et al., 2007. Development and characterization of gemcitabine-resistant pancreatic tumor cells. Ann. Surg. Oncol. 14 (12), 3629–3637.
Shao, Y., Sang, J., Fu, J., 2015. On human pluripotent stem cell control: the rise of 3D bioengineering and mechanobiology. Biomaterials 52, 26–43.
Shibue, T., Weinberg, R.A., 2017. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nat. Rev. Clin. Oncol (advance online publication).
Siegel, R.L., Miller, K.D., Jemal, A., 2017. Cancer statistics, 2017. CA. Cancer J. Clin. 67 (1), 7–30.
Spratlin, J., et al., 2004. The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. Clin. Cancer Res. 10 (20), 6956–6961.
Steinwachs, J., et al., 2016. Three-dimensional force microscopy of cells in biopolymer networks. Nat. Meth. 13 (2), 171–176.
Style, R.W., et al., 2014. Traction force microscopy in physics and biology. Soft Matter 10 (23), 4047–4055.
Taga, T., et al., 2002. αv-Integrin antagonist EMD 121974 induces apoptosis in brain tumor cells growing on vitronectin and tenascin. Int. J. Cancer 98 (5), 690–697.
Tammi, R.H., et al., 2008. Hyaluronan in human tumors: pathobiological and prognostic messages from cell-associated and stromal hyaluronan. Sem. Cancer Biol. (Elsevier) 288–295.
Tan, J.L., et al., 2003. Cells lying on a bed of microneedles: an approach to isolate mechanical force. Proc. Natl. Acad. Sci. 100 (4), 1484–1489.
Thompson, C.B., et al., 2010. Enzymatic depletion of tumor hyaluronan induces antitumor responses in preclinical animal models. Mol. Cancer Ther. 9 (11), 3052–3064.
Topalovski, M., Brekken, R.A., 2016. Matrix control of pancreatic cancer: new insights into fibronectin signaling. Cancer Lett. 381 (1), 252–258.
Trepat, X., et al., 2009. Physical forces during collective cell migration. Nat. Phys. 5 (6), 426.
Vennin, C., et al., 2017. Transient tissue priming via ROCK inhibition uncouples pancreatic cancer progression, sensitivity to chemotherapy, and metastasis. Scie. Trans. Med. 9 (384) eaai8504.
Von Hoff, D.D., et al., 2013. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. New Engl. J. Med. 369 (18), 1691–1703.
Wang, Z., et al., 2009. Acquisition of epithelial-mesenchymal transition phenotype of gemcitabine-resistant pancreatic cancer cells is linked with activation of the notch signaling pathway. Cancer Res. 69 (6), 2400–2407.
Wang, R., et al., 2014. Gemcitabine resistance is associated with epithelial-mesenchymal transition and induction of HIF-1α in pancreatic cancer cells. Curr. Cancer Drug Targets 14 (4), 407–417.
Wang, O.H., et al., 2016. Prognostic and functional significance of MAP4K5 in pancreatic cancer. PLoS One 11 (3), 12325–12330 e0152300.
Warren, K.M., Islam, M.M., LeDuc, P.R., Steward Jr, R., 2016. 2D and 3D mechanobiology in human and nonhuman systems. ACS Appl. Mater. Interfaces 8 (34), 21869–21882.
Watt, J., Kocher, H.M., 2013. The desmoplastic stroma of pancreatic cancer is a barrier to immune cell infiltration. Oncoimmunology 2 (12), e26788.
Whatcott, C.J., et al., 2015. Desmoplasia in primary tumors and metastatic lesions of pancreatic cancer. Clin. Cancer Res. 21 (15), 3561–3568.
Wijdeven, R.H., Pang, B., Assaraf, Y.G., Neefjes, J., 2016. Old drugs, novel ways out: drug resistance toward cytotoxic chemotherapeutics. Drug Resist. Updat. 28, 65–81.
Xu, Z., Pothula, S.P., Wilson, J.S., Apte, M.V., 2014. Pancreatic cancer and its stroma: a conspiracy theory. World J. Gastroenterol. 20 (32), 11216–11229.
Zhang, W., et al., 2014. Downstream of mutant KRAS, the transcription regulator YAP is essential for neoplastic progression to pancreatic ductal adenocarcinoma. Sci. Signal. 7 (324), ra42.
Zhitomirsky, B., Assaraf, Y.G., 2016. Lysosomes as mediators of drug resistance in cancer. Drug Resist. Updat. 24, 23–33.
van Hoorn, H., et al., 2014. The nanoscale architecture of force-bearing focal adhesions. Nano Lett. 14 (8), 4257–4262.