1521-0111/90/6/744–754$25.00
MOLECULAR PHARMACOLOGY

http://dx.doi.org/10.1124/mol.116.106765
Mol Pharmacol 90:744–754, December 2016

MINIREVIEW

The 2016 John J. Abel Award Lecture: Targeting the Mechanical Microenvironment in Cancer

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Received September 2, 2016; accepted October 13, 2016

ABSTRACT

Past decades of cancer research have mainly focused on the role of various extracellular and intracellular biochemical signals on cancer progression and metastasis. Recent studies suggest an important role of mechanical forces in regulating cellular behaviors. This review first provides an overview of the mechanobiology research field. Then we specially focus on mechanotransduction pathways in cancer progression and describe in detail the key signaling components of such mechanotransduction pathways and extracellular matrix components that are altered in cancer. Although our understanding of mechanoregulation in cancer is still in its infancy, some agents against key mechanoregulators have been developed and will be discussed to explore the potential of pharmacologically targeting mechanotransduction in cancer.

Introduction

The role of the tumor microenvironment in cancer progression has recently become a focal point of research in the cancer biology field. From the role of immune cells, to cancer-associated fibroblasts, to the extracellular matrix (ECM), all of these factors are shown to have profound effects on tumor growth, local invasion, intravasation, extravasation, metastatic seeding, and outgrowth. The focus of this review is on the role of ECM, particularly mechanical properties of the ECM, in tumorigenesis and progression, and the emerging cancer therapeutic targets that this relatively new field is bringing forward.

The process by which cells sense mechanical cues in their environment and transform them into biochemical signals is called mechanotransduction. These mechanical cues range from changes in ECM rigidity, to fluid shear stress, to cell stretch or intracellular strain or intercellular compression. Initially, mechanotransduction was studied in a small number of specialized cells that had a clear need to sense and transduce these types of signals, such as sensory cells. The classic example of this is hair cells of the inner ear, which sense mechanical forces such as sound waves, gravity, and pressure, and transduce them into biochemical signaling pathways to generate hearing sensation. These hair cells have specialized structures called stereocilia that are attached at their tips by extracellular filaments called tip linkers. When stereocilia are deformed by mechanical forces, these tip linkers are stretched and open the attached ion channels on the stereocilia, causing an influx of ions to initiate downstream signaling (Vollrath et al., 2007). Other types of sensory cells, such as proprioception and touch, have similar underlying mechanotransduction signaling mechanisms (Eberl et al., 2000; Syntichaki and Tavernarakis, 2004). This early example of mechanotransduction provides a good example for one of the essential components of mechanotransduction: mechanically induced protein conformational change.

Whereas the study of mechanotransduction at its beginning was focused on sensory cells and organs, it has since been discovered that mechanotransduction plays an important role in the morphology and physiology of a variety of tissues: the heart and vasculature are affected by the pressure and shear

This work was supported by National Institutes of Health National Cancer Institute [Grants 1RO1CA168689, 1R01CA174869, and 1R01CA206880]; Department of Defense Breast Cancer Program [Grant W81XWH-13-1-0132 to J.Y.]; and National Institutes of Health [Predoctoral Training Grant 5T32GM007752 to H.E.M.].

J.Y. is the 2016 recipient of the John J. Abel Award in Pharmacology given by ASPET. This work was previously presented at the following lecture: Jing Yang (2016) Epithelial-Mesenchymal Plasticity in Carcinoma Metastasis. *ASPET Annual Meeting*; 2016 Apr 4; San Diego, CA.

dx.doi.org/10.1124/mol.116.106765.

ABBREVIATIONS: ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GAG, glycosaminoglycan; HA, hyaluronan; HAS, HA synthase; HMW-HA, high-molecular-weight HA; HYAL, hyaluronidase; IFP, interstitial fluid pressure; LMW-HA, low-molecular-weight HA; LOX, lysyl oxidase; LOXL, LOX-like; Pa, pascal; PEGPH20, PEGylated human recombinant PH20 hyaluronidase; PG, proteoglycan; PI3K, phosphoinositide 3-kinase; Pyk2, protein tyrosine kinase 2β; ROCK, rho-associated protein kinase; TAZ, Tafazzin; TEAD, TEA domain; TGF-β, transforming growth factor-β; YAP, yes-associated protein.

stress of flowing blood (Gimbrone et al., 2000; Garcia-Cardeña et al., 2001; Li et al., 2005; Haga et al., 2007), the lungs are influenced by the distention and contraction of breathing and the changing mechanical stresses it causes (Wirtz and Dobbs, 2000), and bone is affected by gravity and compressive forces (Burger and Klein-Nulend, 1999). On the cellular level, mechanical forces regulate the behavior of many, if not all, cell types, including myocytes, endothelial cells, and vascular smooth muscle cells. For example, naive mesenchymal stem cells can be driven to differentiate into different cell types depending on the rigidity of the underlying matrix—differentiating into neurogenic cells on softer matrices that resemble the rigidity of the brain, into myocytes on stiffer matrices that are similar to that of muscle tissues, and osteoblasts on very rigid matrices that mimic the stiffness of bone (Engler et al., 2006).

Mechanotransduction Mechanisms

Recent studies began to reveal how mechanical forces are interpreted by cells to generate cellular responses. At the most basic level, a mechanotransduction pathway starts with the sensing of mechanical stimuli through force-induced conformation change of mechanically sensitive molecules, which leads to activation of downstream biochemical signaling pathways, effectively relating a mechanical cue into a biochemical signal. Although a few of these mechanically sensitive molecules have been discovered, a large number of them are likely still to be identified. Based on currently known mechanical sensors, these conformation changes usually occur in three modes: force-induced opening of ion channels, force-induced unfolding of proteins exposing cryptic binding sites for other proteins, and force-induced alteration in enzymatic activity (Wang et al., 2005; Sawada et al., 2006).

The first cases of mechanosensitive ion channels were discovered in bacteria, such as the mechanosensitive channel of large conductance and mechanosensitive channel of small conductance channels that open in response to membrane stretch in *Escherichia coli* (Martinac et al., 1987; Sukharev et al., 1994; Sotomayor and Schulten, 2004). These mechanically sensitive channels are also prevalent in sensory cells, such as the hair cells discussed above. The mechanosensory mechanisms in nonsensory cell types have proven to be more complicated and involve a wider variety of protein structures. The focal adhesion complex, serving many roles in the adhesion and migration of cells, has also been shown to be a major mechanosensing structure. Its key components, integrins, are transmembrane proteins that bind to various ECM proteins to sense mechanical properties of the matrix and also associate with a number of intracellular proteins (Jaalouk and Lammerding, 2009). Among them, talin and vinculin both bind actin, serving as a link between integrins and the actomyosin cytoskeleton network (Humphrey et al., 2014). Actin, nonmuscle myosin, and various other associated proteins, which make up the actomyosin network in a cell, transmit mechanical loads within the cell (Jaalouk and Lammerding, 2009). Importantly, some of these adaptor proteins act as mechanosensory molecules through conformational changes. Talin, for example, exhibits force-induced unfolding and exposure of cryptic binding sites (Elosegui-Artola et al., 2016). This unfolding occurs in response to a threshold of force, usually in the form of matrix rigidity, needed to induce subsequent activation of downstream signaling pathways leading to focal adhesion reinforcement.

In addition to the adaptor proteins, there are a plethora of focal adhesion proteins that are recruited to this complex upon formation, and this review does not intend to address each of them in detail. One essential protein that will become important later, when discussing mechanosensing targets in cancer, is focal adhesion kinase (FAK). This kinase is recruited to focal adhesions after integrin engagement, and, upon autophosphorylation, creates Src homology 2 docking sites for Src kinase, which then recruits other adhesion proteins such as p130Cas, and paxillin (Nojima et al., 1995; Panetti, 2002; Sawada and Sheetz, 2002; Hanks et al., 2003; Sawada et al., 2006). This in turn, leads to activation of guanine nucleotide exchange factors for Rho and subsequent activation of Rho effectors such as rho-associated protein kinase (ROCK), thus increasing myosin activity and actomyosin (Pasapera et al., 2010; Lessey et al., 2012; Carisey et al., 2013). Notably, p130Cas has also been shown to undergo force-induced conformation change (Sawada et al., 2006; del Rio et al., 2009; Moore et al., 2010; Margadant et al., 2011; Wang et al., 2011; Hotta et al., 2014), and Src has been shown to undergo force-induced kinase activation (Arias-Salgado et al., 2003; Wang et al., 2005; Sawada et al., 2006).

Although these, and several other effectors, have been shown to change conformation, or become activated in response to force, it is important to note that mechanical sensing differs significantly among various cell types. Most published studies on focal adhesions and matrix rigidity were conducted in fibroblasts, which respond to a certain rigidity level. This threshold for sensing rigidity seems to be a common theme among different cell types, whether through focal adhesions or not; however, the threshold force differs depending on the cell type. Fibroblasts and endothelial cells appear to activate their cytoskeleton and increase spreading at about 3000 pascals (Pa; unit of force measurement), whereas preosteocytes do not respond until about 60,000 Pa. Neutrophils, in contrast, respond to rigidities as low as 2 Pa (Kong et al., 2005; Yeung et al., 2005), and mammary epithelial cells start to change morphology at about 300–500 Pa (Paszek et al., 2005). This indicates that there are a variety of different threshold-sensing mechanisms, the majority of which have yet to be identified.

One additional mechanosensing mechanism that is largely in its infancy involves the force-induced nuclear organization. What is known is that this involves connecting the cytoskeleton to the nucleus through Nesprin molecules. Nesprins bind to both cytoskeleton and nuclear membrane proteins, which then interact with nuclear envelope proteins such as lamins to form stable structures with DNA. This link between the cytoskeleton and the nucleus transmits mechanical cues to changes in chromatin structure or movement of DNA and chromatins (Haque et al., 2006).

Mechanotransduction in Cancer

In addition to regulating normal physiology, mechanotransduction is recognized to play important roles in tumor progression. The extracellular environment of tumors, or tumor stroma, constantly changes as tumors progress. The ECM around tumors is found to stiffen progressively in a variety of human cancer types. Perhaps the best example is in breast

cancer, which is often first detected by direct palpitation due to its increased stiffness compared with surrounding tissues. The presence of a fibrotic focus, which is an accumulation of collagen and fibroblasts, is correlated with an increase in metastatic disease and a decrease in recurrence-free survival in patients (Colpaert et al., 2001; Boyd et al., 2002; Hasebe et al., 2002; Mujtaba et al., 2013). In three-dimensional cultures mimicking stiffness changes during breast cancer progression, altering ECM rigidity could induce an invasive, malignant phenotype in mammary epithelial cells (Paszek et al., 2005; Levental et al., 2009). Although cells normally exist in their physiologic environment with certain rigidities, pressure, and strain, alterations in this environment can aberrantly activate certain mechanotransduction pathways, leading to a variety of tumorigenic processes such as sustained proliferation, resistance to cell death and epithelial-mesenchymal transition (EMT), invasion, and metastasis.

One of the hallmarks of cancer is sustained proliferation. Mechanical cues are shown to increase cell proliferation, and, when aberrantly activated by a deregulated extracellular environment, can facilitate cancer development. Mechanistically, increasing stiffness can promote growth factor signaling and lead to enhanced proliferation. In a glioblastoma model, increased ECM stiffness enhanced activation and expression of epithelial growth factor receptor and its downstream mitogenic factors such as phosphoinositide 3-kinase (PI3K) and RAC-α serine/threonine-protein kinase (Umesh et al., 2014). Increases in rigidity could also promote transition through G_1/S phases of the cell cycle. In mouse embryonic fibroblasts, an increase in FAK activation and p130Cas signaling led to activation of extracellular signal-regulated kinase (ERK) and PI3K signaling and subsequently Rac, which induced cyclin D1 to increase cell proliferation (Chambard et al., 2007; Provenzano et al., 2008; Pylayeva et al., 2009; Provenzano and Keely, 2011; Bae et al., 2014). Matrix stiffening is also shown to induce the expression of microRNAs, such as miR18a to inhibit the expression of tumor suppressor phosphatase and tensin homolog. This increases PI3K/RAC-α serine/threonine-protein kinase activities and cell growth and survival (Mouw et al., 2014). Transcription coactivators yes-associated protein (YAP)/Tafazzin (TAZ), which promote cell growth and inhibit apoptosis, are also sensitive to mechanical cues from the ECM. Increasing matrix stiffness promotes YAP/TAZ nuclear localization to drive proliferation gene expression and overcome growth suppression (Dupont et al., 2011).

In addition to promoting cell proliferation, alterations in ECM mechanics can also enhance tumor growth through promoting resistance to cell death. Transforming growth factor-β (TGF-β) is known to induce both apoptosis and EMT, depending on biologic contexts. When cells are cultured on a soft, compliant ECM, TGF-β induces apoptosis; however, on stiff ECM, TGF-β switches its role to promote EMT, leading to a decrease in cell death and an increase in invasiveness (Leight et al., 2012). Increased rigidity can also promote integrin-mediated cell survival (Paszek et al., 2014). Adhesion to ECM inactivates proapoptotic molecule Bcl-2–associated X and induces antiapoptotic gene B cell lymphoma 2 (Ruoslahti and Reed, 1994; Frisch et al., 1996; Gilmore et al., 2000). ECM stiffening can also promote anchorage-independent cell survival through Rac and resistance to anoikis (cell death resulting from lack of attachment) through FAK signaling (Zahir et al., 2003; Zhang et al., 2004).

As mentioned earlier, through various mechanisms, increased rigidity appears to induce a malignant phenotype (Paszek et al., 2005; Leight et al., 2012). Several recent studies show that increases in rigidity can promote invasion and metastasis—by enhancing the activity of matrix metalloproteinases to aid in degradation and invasion through ECM (Haage and Schneider, 2014), via promoting invadopodia formation for ECM degradation (Parekh et al., 2011), and by inducing EMT. Two mechanistic studies directly linked increasing matrix stiffness to two major EMT-inducing transcription factors. Our study found that increasing matrix rigidities could induce EMT and tumor invasion and metastasis in mammary tumor cells by activating the EMT-inducing transcription factor Twist1. Increasing rigidities led to nuclear translocation of Twist1 by releasing Twist1 from its cytoplasmic anchor proteins G3BP2 (Wei et al., 2015). A recent study found that increasing matrix stiffness could promote protein stability of another EMT transcription factor Snail1 through enhanced collagen/discoidin domain-containing receptor 2 binding. Activation of discoidin domain-containing receptor stimulated ERK activity via Src, and ERK subsequently phosphorylates Snail1, leading to enhanced nuclear accumulation and decreased ubiquitination (Zhang et al., 2013). Together, these studies provide direct links between matrix stiffening and the molecular machinery of tumor invasion and metastasis.

Interestingly, ovarian cancer cells were shown to present opposite responses to matrix rigidities—these cancer cells preferentially adhere to softer ECM and present an enhanced invasive phenotype when cultured on softer substrates through RHO/ROCK signaling pathways (McGrail et al., 2014). This supports the notion that multiple mechanosensing mechanisms exist among different cell types possibly due to the different mechanical environments of their tissues of origin. The same alterations in ECM could yield vast different biologic responses; thus, targeting mechanotransduction pathways needs to be tailored for specific cancer types.

Currently, our understanding on how mechanical signals are sensed and transmitted to generate specific cellular responses is still rudimental. In most cases, the mechanotransduction pathway linking the mechanical sensors all the way to the transcriptional or translational responses in response to specific mechanical cues is far from completely defined. As described above, more and more cellular studies are carried out under physiologic stiffness of the tissue of origin, instead of simply on plastic dishes with extremely high rigidities out of physiologic ranges. Future studies incorporating physical properties of matrix into experimental conditions could uncover many hidden mechanical regulatory mechanisms for therapeutic targeting.

Targeting Mechanotransduction Pathways in Cancer

Two major components of mechanotransduction pathways are primary targets in cancer: one is through targeting biomechanical proteins activated by mechanotransduction, or the other one is through targeting the altered ECM components themselves. Targeting mechanotransduction pathways has largely focused on affecting the well-established

mechanoactivators such as integrins, FAK, Src, and the obvious mechanotransducers such as RHO/ROCK.

Targeting Integrin-Mediated Mechanosensing in Cancer. The rationale for targeting integrins came from early studies by Humphries et al. (1986) in the 1980s, in which they used RGD peptides to block large subsets of integrins, and found that this could reduce tumor cell invasion in vitro and metastasis in vivo in mouse models. Since then, various targeting strategies for integrins have been tested, such as utilizing synthetic peptides containing RGD sequences or other integrin-binding sequences, nonpeptide molecules that mimic RGD sequences, or integrin-binding proteins called disintegrins that are isolated from viper snake venom and contain RGD sequences (Curley et al., 1999). The first blocking antibody developed is a humanized version of the LM609 $\alpha5\beta3$ antibody called etaracizumab. Whereas it initially showed low toxicity in phase 1 and 2 trials, it was eventually found to present no benefit than standard chemotherapy (Gutheil et al., 2000; McNeel et al., 2005; Delbaldo et al., 2008; Hersey et al., 2010). Cilengitide, a cyclic RGD peptide, was developed to selectively block $\alpha5\beta3$ and $\alpha5\beta5$ on blood vessels to block angiogenesis, and showed promise in phase 2 trials for lung and prostate cancer and glioblastoma, but recent phase III trials showed no added benefits to standard therapies for glioblastoma (Beekman et al., 2006; Nabors et al., 2007; MacDonald et al., 2008; Reardon et al., 2008; Desgrosellier and Cheresh, 2010).

A couple of studies have focused on targeting $\alpha5\beta1$ specifically. The drug ATN-161 was derived from a synergy sequence PHRSN, which is a second recognition motif required for this integrin complex to interact with fibronectin (Danen et al., 1995). This molecule was found to inhibit tumor growth and metastasis in animal models (Livant et al., 2000; Khalili et al., 2006), and, in phase 1 trials, there were no toxicities and one third of patients had a prolonged stable disease state (Cianfrocca et al., 2006). This is further supported by another $\alpha5\beta1$ target, volociximab, which also showed low toxicity in phase 1 trials and showed a modest amount of disease stabilization (Ricart et al., 2008).

The low efficacy of targeting integrins could be due to the broad functions of integrins in multiple cell types in tumors. For example, treatment with low-dose RGD peptides can actually enhance vascular endothelial growth factor–mediated angiogenesis and tumor growth, which suggests that, in certain contexts, certain integrins may be acting in a tumor-suppressive manner, and blocking them has an undesired effect (Reynolds et al., 2009).

Targeting the Mechanosensing Molecule FAK in Cancer. A fair amount of effort has been put into targeting FAK, the protein downstream of integrins, to block activation of certain protumorigenic mechanosensing pathways that are activated upon enhanced collagen deposition, cross-linking, or other mechanical stresses. The FAK inhibitors under development all present dual specificities for FAK and a kinase called protein tyrosine kinase 2β (Pyk2), which is a homolog that can compensate for FAK. Two early FAK inhibitors, PF-573228 and NVP-TAC544, developed by Pfizer and Novartis, respectively, both inhibit FAK specifically, with little activity against Pyk2, and subsequently failed to inhibit proliferation or induce apoptosis in tumor cells (Slack-Davis et al., 2007; Lim et al., 2008). However, both served as backbones for future development of dual-specific inhibitors. PF-562271, one of the first clinically available FAK inhibitors, is an ATP-competitive reversible inhibitor of the catalytic activity for FAK and Pyk2. In several preclinical mouse xenograft models, this inhibitor led to dose-dependent tumor regression (Roberts et al., 2008). Another study in 2011 showed that the tumor-suppressive effects of this drug on pancreatic cancer cells in vitro are through inhibiting tumor cell migration, invasion, and proliferation, and in vivo is through reducing tumor growth, invasion, and metastasis in an orthotopic murine model (Stokes et al., 2011). In a phase 1 clinical trial on head and neck, prostate, and pancreatic tumors, there were no significant adverse effects, and about one third of the patients had stable disease (Schultze and Fiedler, 2010; Infante et al., 2012).

There have been several subsequent FAK inhibitors developed, most in various stages of preclinical testing. One such inhibitor, NVP-TAE226, showed excellent antitumor effects in preclinical studies in vitro and in animal studies against glioma (Liu et al., 2007; Shi et al., 2007), breast (Golubovskaya et al., 2008) and ovarian cancer (Halder et al., 2007), neuroblastoma (Beierle et al., 2008), esophageal cancer (Wang et al., 2008; Watanabe et al., 2008), and gastrointestinal stromal tumors (Sakurama et al., 2009). However, this compound was also found to inhibit insulin growth factor and insulin receptor, causing impaired glucose metabolism in many animal models, and preventing it from ever entering clinical testing. Recently, preclinical testing of new FAK inhibitors VERSUS-4718 was shown to limit tumor fibrosis and tumor progression in an in vivo model of human pancreatic ductal adenocarcinoma (Jiang et al., 2016). VERSUS-4718 is in current phase 1 trials as a single agent in solid tumors or in combination with chemotherapies. These results collectively show the promise of FAK inhibitors in preclinical and clinical testing.

Targeting Downstream Mechanotransducers Src, Rho, ROCK, and YAP/TAZ in Cancer. Additional efforts aim to target more downstream components of mechanotransduction pathways, such as Src, Rho GTPases, and YAP/TAZ signaling, all of which are intimately linked with each other in mechanosensing. Rho, together with Rho kinase ROCK, regulates myosin light chain phosphorylation through inhibiting myosin phosphatases. Rho GTPases also activate YAP/TAZ by inhibiting their phosphorylation and subsequently promoting the accumulation of these transcription activators in the nucleus (Dupont et al., 2011). Nuclear YAP/TAZ act as transcriptional coactivators for the TEA domain (TEAD) family of transcription factors, which induces the expression of apoptosis inhibitors and proliferative genes (Vassilev et al., 2001).

ROCK inhibitor Fasudil inhibited tumor progression in various tumor models, including small cell lung cancer and glioblastoma (Ying et al., 2006; Deng et al., 2010; Yang et al., 2012). Some tumors present increased Rho activity, leading to more cytoskeletal tension; however, some reports also found that there is decreased Rho activity in certain solid tumors (Horiuchi et al., 2003; Sahai and Marshall, 2003). One of the main approaches for targeting Rho is through the mevalonate pathway, a metabolic cascade that generates geranylgeranyl moieties needed for post-translational modification and membrane localization of Rho-GTPases. A key enzyme in this pathway is 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, which is inhibited by statins. Statin treatment caused cytoplasmic relocalization and inhibition of YAP/TAZ in

several cancer types and a reduction of cancer stem cell growth and tumor xenografts (Sorrentino et al., 2014; Wang et al., 2014; Li et al., 2015). Bisphosphonates and geranylgeranyl transferase 1 inhibitors also target the mevalonate pathways and have similar effects on inhibiting YAP and TAZ (Sorrentino et al., 2014; Mi et al., 2015).

Additionally, a direct inhibitor of YAP/TAZ, verteporfin, could inhibit the interaction between YAP and its binding transcription factor TEAD, thus suppressing the downstream YAP/TEAD signaling in retinoblastoma (Liu-Chittenden et al., 2012; Brodowska et al., 2014; Wang et al., 2015). Src inhibitors, such as Dasatinib, have shown some effect in tumor inhibition by itself (Zhang and Yu, 2012). However, monotherapy phase II trials for Src inhibitors have not been very promising, with modest effects in breast, prostate, and melanoma (Mayer and Krop, 2010), and no benefit for several other cancer types such as small cell lung cancer (Miller et al., 2010). However, Src inhibition through Dasatinib has been shown to oppose YAP/TAZ transcriptional effects in vitro and in vivo (Rosenbluh et al., 2012; Calvo et al., 2013). When Dasatinib is given in combination with statin treatment to inhibit Rho, it appears to have a much more significant effect on the downstream YAP/TAZ activity (Taccioli et al., 2015). This indicates that perhaps when attempting to target downstream effectors of mechanosensing pathways, it could be beneficial to hit multiple effectors at once.

The inhibitors discussed in this section indicate the promise of targeting mechanotransduction in cancer therapies. These inhibitors, their targets, and their efficacy are summarized in Table 1. It is important to note that many of the mechanotransduction proteins targeted in this study also play important roles in response to nonmechanical cues; therefore, the therapeutic responses are a combined effect from inhibiting all the biologic functions involved. The majority of these inhibitors are still limited to preclinical testing, although the few that have entered clinical trials seem to show promise in terms of disease stabilization. However, there are a few inhibitors that, although they showed promise in preclinical study, had little effect on tumors in clinical trials, which suggests that inhibition of a single mechanosensing molecule is not sufficient to yield significant antitumor effects. Recent efforts have begun to combine therapies, such as the Dasatinib and statin double treatment, or combine these targeted therapies with chemotherapy to potentiate their effects.

ECM Components in Mechanotransduction and Cancer

An alternative approach for targeting mechanotransduction is to target the altered ECM components in the tumor microenvironment. The ECM is a complex network of molecules secreted by cells that forms the connective tissue between cells and provides structural support for organs. In addition to its structural role, the ECM provides many biochemical and mechanical cues to cells to regulate their behaviors, and vice versa. The ECM is comprised of proteins, proteoglycans (PGs), and glycosaminoglycans (GAGs), including collagen (types I, III, and IV), fibronectin, elastin, laminin, hyaluronan (HA), and a variety of other, more tissue-specific, types of ECM molecules (Whittaker et al., 2006; Ozbek et al., 2010). The mechanical properties of the ECM largely depend on elastin fibers, which provide extensibility and resilience to the matrix (Arribas et al., 2006; Kielty, 2006; Humphrey et al., 2014), and collagen fibrils and associated GAGs and PGs, which provide material stiffness and strength (Humphrey et al., 2014). PGs and GAGs are highly negatively charged and can attract a lot of water, thus contributing to compressive stiffness (Humphrey et al., 2014). Two particularly important molecules for mechanotransduction, especially in the targeting of mechanotransduction in cancer, collagen, and HA, are discussed in more detail below.

Collagen is the most abundant protein in the human body, and there are over 25 different types of collagen, the most common being fibrillary types I and III (Humphrey et al., 2014). Collagens are built from trihelical molecules, to fibrils to fibers, and are further modified through reorientation, cross-linking, and turnover. Collagen fibril cross-linking is carried out by the lysyl oxidase (LOX) family of enzymes to produce collagen fibers (Kagan and Li, 2003; Vadasz et al., 2005; Kim et al., 2011). Reorganization into fibers increases the density and rigidity of ECM. The presence of highly cross-linked and long straight collagen fibers in tumors is associated with increases in metastasis in breast cancer patients (Barker et al., 2012; Cox et al., 2013), and expression of LOX enzymes is also found to be increased in various human cancer types (Barker et al., 2012). Additionally, organization of these fibers also facilitates invasive migration along these fibers (Egeblad et al., 2010; Zhang et al., 2013).

HA is a GAG that makes up a significant portion of the ECM in a variety of tissues. HA is the only GAG comprised of unsulfated disaccharides without a proteoglycan core (Gandhi and Mancera, 2008). HA is synthesized by HA synthases (HAS1–3), which produce high-molecular-weight HA (HMW-HA) (Itano and Kimata, 2002). HMW-HA can then be degraded by hyaluronidases (HYAL 1,2,3 or PH20) or reactive oxygen species into low-molecular-weight HA (LMW-HA) or oligo HA (Heldin et al., 2013). HA is a substrate for cell adhesion (Aruffo et al., 1990; Miyake et al., 1990), and CD-44 is the most well-characterized receptor for HA (Aruffo et al., 1990). Cells experiencing mechanical pressure are induced to synthesize HA (Simpson and Lokeshwar, 2008).

The cellular response to HA depends on the size (HMW-HA versus LMW-HA) and concentration of HA. Increased deposition of HA is observed in many solid cancers (Sironen et al., 2011); however, this response seems to depend on HMW-HA or LMW-HA and the relative expression of HASs and HYALs. HMW-HA retention in prostate tumor cell lines correlates with metastatic potential in mice (Simpson et al., 2001), and prostate tumors that produce large quantities of endogenous HA polymers are more metastatic than cells overexpressing HYAL-1, suggesting that perhaps LMW-HA has more potent effect on metastasis (Patel et al., 2002). This notion is further supported by West and Kumar (1989), who showed that expression of large quantities of HMW-HA was actually antiproliferative, and antiangiogenic, suppressing growth of tumor cells and vascular endothelial cells. Bharadwaj et al. (2009) addressed this through examining the effect of HAS and HYALs on tumorigenicity and metastasis in prostate cancer in mice. They found that HAS overexpression led to less tumor growth and metastasis than control tumors; however, cells expressing both HAS and HYAL are much more tumorigenic and metastatic than controls. Together, these data indicate that HYAL expression

TABLE 1

Targeting mechanotransduction pathways in cancer

This table describes several inhibitors that target various mechanosensing molecules and the results from both preclinical models and clinical trials where applicable. Relevant references are also listed for each inhibitor.

Inhibitor	Target of Inhibitor	Effectiveness of Inhibitor	Relevant References
RGD peptides	Integrins (nonspecific)	Reduced invasion in vivo and in vitro in mouse models	Humphries et al., 1986; Curley et al., 1999
Etaracizumab	Integrin $\alpha5\beta3$ blocking antibody	Low toxicity phase 1 and 2, no benefit above standard chemotherapy	Gutheil et al., 2000; McNeel et al., 2005; Delbaldo et al., 2008; Hersey et al., 2010
Cilengitide	Cyclic RGD peptide specific for integrins $\alpha5\beta3$ and $\alpha5\beta5$ on blood vessels to block angiogenesis	Promising phase 1 and 2 trials in lung and prostate cancer and glioblastoma; phase 3 trials showed no benefit to standard chemotherapy in glioblastoma	Beekman et al., 2006; Nabors et al., 2007; MacDonald et al., 2008; Reardon et al., 2008; Desgrosellier and Cheresh, 2010
ATN-161	Integrin $\alpha5\beta1$ and fibronectin interaction through targeting $\alpha5\beta1$ PHRSN sequence	Inhibited tumor growth and metastasis in animal models; phase 1 trials showed no toxicity and 1/3 patients had prolonged stable disease	Danen et al., 1995; Livant et al., 2000; Cianfrocca et al., 2006; Khalili et al., 2006
Volociximab	Monoclonal blocking antibody for integrin $\alpha5\beta1$	Low toxicity in phase 1 trials, some disease stabilization	Ricart et al., 2008
PF-573228	ATP-competitive selective FAK inhibitor	No inhibition of proliferation or apoptotic induction in tumor cells	Slack-Davis et al., 2007; Lim et al., 2008
NVP-TAC544	ATP-competitive selective FAK inhibitor	No inhibition of proliferation or apoptotic induction in tumor cells	Lim et al., 2008
PF-562271	FAK and Pyk2 ATP-competitive reversible inhibitor	Dose-dependent tumor regression in mouse xenograft models; effective against pancreatic cancer growth and invasion in vivo and in vitro; phase 1 trials in head and neck, prostate, and pancreatic cancer showed no adverse effects and 1/3 patients with stable disease	Roberts et al., 2008; Schultze and Fiedler, 2010; Stokes et al., 2011; Infante et al., 2012
NVP-TAE226	ATP-competitive FAK and Pyk2 inhibitor	Effective in mouse preclinical studies in glioma, breast, and ovarian cancer, neuroblastoma, esophageal cancer, and GIST; also inhibited insulin receptors and led to altered glucose metabolism in preclinical testing	Halder et al., 2007; Liu et al., 2007; Shi et al., 2007; Beierle et al., 2008; Golubovskaya et al., 2008; Wang et al., 2008; Watanabe et al., 2008; Sakurama et al., 2009
VERSUS-6063	FAK and Pyk2 inhibitor	Phase 1 trial as a single agent and in combination with chemotherapy	Jiang et al., 2016
VERSUS-4718	FAK and Pyk2 inhibitor	Limited tumor fibrosis and progression in preclinical testing for pancreatic ductal adenocarcinoma; phase 1 trial as a single agent and in combination with chemotherapy	Jiang et al., 2016
Fasudil	ROCK inhibitor	Inhibited tumor progression in preclinical mouse tumor models for small cell lung cancer and glioblastoma	Ying et al., 2006; Deng et al., 2010; Yang et al., 2012
Statins	HMG-CoA reductase inhibitor (indirectly inhibits RHO)	YAP/TAZ inhibition and cytoplasmic relocalization; reduction in cancer stem cell growth and tumor xenograft models	Sorrentino et al., 2014; Wang et al., 2014; Li et al., 2015
Verteporfin	YAP/TAZ inhibitor	Inhibits YAP-TEAD binding and suppresses downstream signaling in retinoblastoma	Liu-Chittenden et al., 2012; Brodowska et al., 2014; Wang et al., 2015
Dasatinib	Src inhibitor	Monotherapy phase 2 trials show some effect in breast and prostate cancer and melanoma, and no benefit in other cancer types such as small cell lung cancer; combination in with statin treatment has a greater effect on downstream YAP/TAZ activity	Mayer and Krop, 2010; Miller et al., 2010; Rosenbluh et al., 2012; Calvo et al., 2013; Taccioli et al., 2015

HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; GIST, gatrointestinal stromal tumor.

and the subsequent degradation of HMW-HA to LMW-HA are important in tumor progression, which will become important in targeting the HA pathway in cancer discussed later in this review.

Another important aspect with regard to HA is how its presence affects the overall mechanical properties of the ECM. Matrices containing a large amount of HA tend to be less organized and more gel-like (Rooney and Kumar, 1993). Increases in the HA content of the ECM can also cause a significant increase in colloid osmotic pressure, and subsequently an increase in interstitial fluid pressure (IFP). This poses challenges for cancer drug delivery—high IFP can cause tumor vessels to collapse, limiting penetration of therapeutics that need the vasculature for transmission (Heldin et al., 2004; Thompson et al., 2010; Provenzano et al., 2012; Jacobetz et al., 2013).

Targeting the ECM Components in Cancer

Targeting Collagen Synthesis and Modification in Cancer. With respect to targeting the ECM components, most therapies are designed to inhibit the enzymes responsible for creating the stiffer tumor-associated matrices, including the LOX family of enzymes for cross-linking collagen or the hyaluronidase family for digest HA. The LOX family of enzymes, which includes LOX and LOX-like (LOXL) proteins 1–4, are secreted copper-dependent amine oxidases, and their primary function is covalently cross-linking collagens, which

subsequently creates a stiffer ECM (Kagan and Li, 2003; Vadasz et al., 2005; Kim et al., 2011). The level of LOX mRNA and protein increases in various types of cancers, including head and neck squamous cell carcinoma (Erler et al., 2006), breast (Kirschmann et al., 2002; Erler et al., 2006), colorectal (Baker et al., 2011, 2013), and prostate (Lapointe et al., 2004). LOXL2 promotes tumor cell survival and chemoresistance; regulates cell adhesion, motility, and invasion; and its expression correlates with poor prognosis and decreased survival (Kirschmann et al., 2002; Grützmann et al., 2003; Fong et al., 2007; Offenberg et al., 2008; Peinado et al., 2008; Peng et al., 2009; Barry-Hamilton et al., 2010; Rückert et al., 2010; Sano et al., 2010; Barker et al., 2011; Moreno-Bueno et al., 2011).

Studies suggest that at least some of the tumorigenic properties of enhanced rigidity are due to the activity of LOX proteins in increasing collagen cross-linking and subsequent rigidity; therefore, targeting this enzyme has attracted a lot of interests. In preclinical studies using β-aminopropionitrile (a LOX inhibitor), functional blocking antibodies against LOX or LOX-specific RNA interference suppressed lung and liver metastasis in tumor xenograft and transgenic mouse models (Erler et al., 2006). In early clinical trials, targeting LOX has shown some mild efficacy. Nonspecific LOXL2 inhibitor diphenylethylenediamine, which binds to intermediary reaction products and prevents cross-linking, allosteric monoclonal inhibitory antibodies, or other LOXL2-targeted antibodies all produced some reduction in primary tumor growth in preclinical studies (Peng et al., 2009; Barry-Hamilton et al., 2010; Barker et al., 2011; Moreno-Bueno et al., 2011). A humanized LOXL2 antibody has begun phase 1 clinical trials (AB0024) (NCT01323933), and a LOXL2-targeting antibody has undergone phase 2 trials in combination with gemcitabine treatment in pancreatic cancer. Although initial studies on late-stage cancer did not yield promising results (Barry-Hamilton et al., 2010), additional study by Miller et al. (2015) showed that this combination therapy was efficacious if administered early. Lastly, some efforts are directed against a different enzyme that functions much upstream in the collagen synthesis pathway, collagen prolyl 4 hydroxylases. This is the enzyme that converts proline to hydroxyproline on collagen, and inhibition of it decreases spontaneous lung metastases in preclinical models (Gilkes et al., 2013; Xiong et al., 2014).

Targeting HA Production and Modification in Cancer. Diverse approaches have been developed to target HA in cancer given the complex roles that LMW-HA and HMW-HA play in cancer. The first method is to directly inhibit HA synthesis or HA binding to its receptor. The 4-MU is a HA synthesis inhibitor and has antitumor activity in preclinical prostate cancer models (Lokeshwar et al., 2010), presumably through depleting UDP-glucuronic acid, a precursor of HA (Kakizaki et al., 2004). Using small oligo HA to block normal (mostly larger) HA signaling through its occupation of HA receptors is shown to inhibit tumor growth in breast and lung cancers, osteosarcoma, and melanoma (Evanko and Vogel, 1990; Urakawa et al., 2012a,b). However, oligo HA also has a tumor-promoting effect in certain cancers such as colorectal cancers (Schmaus et al., 2014a,b), most likely due to distinct roles of different sizes of HA oligos in cancer progression.

As mentioned previously, overexpression of HYAL enzymes (which degrade HMW-HA to LMW-HA) led to increased

TABLE 2

Targeting ECM components in cancer

This table describes the different inhibitors that target the production, modification, and function of the relevant ECM molecules discussed in the section. The effectiveness of these inhibitors both in preclinical models and in clinical trials where applicable is also described. Relevant references are listed for each inhibitor.

Inhibitor	Target of Inhibitor	Effectiveness of Inhibitor	Relevant References
BAPN	LOX inhibitor	Suppressed lung and liver metastasis in preclinical models	Erler et al., 2006
DPEN	Nonspecific Loxl2 inhibitor; prevents collagen cross-linking	Reduced primary tumor growth in preclinical studies	Peng et al., 2009; Barry-Hamilton et al., 2010; Barker et al., 2011; Moreno-Bueno et al., 2011
Loxl2 antibodies	Inhibit Loxl2	Phase 2 trials in combination with gemcitabine in pancreatic cancer ineffective in late stage cancer but efficacious administered early	Barry-Hamilton et al., 2010; Miller et al., 2015
DHB	Inhibits collagen prolyl 4 hydroxylases	Decreased spontaneous lung metastases in preclinical models	Gilkes et al., 2013; Xiong et al., 2014
4-MU	Inhibits HA synthesis by acting as a competitive substrate for UDP-glucuronosyltransferase	Antitumor activity in preclinical prostate cancer models	Kakizaki et al., 2004; Lokeshwar et al., 2010
Small oligo HA	Binds to HA receptors and blocks larger HA molecules	Inhibit tumor growth in breast and lung cancers, osteosarcoma, and melanoma; also has tumor-promoting effects in colorectal cancer	Evanko and Vogel, 1990; Benitez et al., 2011; Urakawa et al., 2012a,b; Schmaus et al., 2014a,b
Sulfated HA	Inhibits HYAL activity	Tumor-inhibitory effects in vitro; inhibited tumor growth in preclinical prostate cancer models	
PEGPH20	Human recombinant PH20 hyaluronidase; degrades HA	In preclinical pancreatic cancer models it inhibited tumor growth, lowered IFP, increased penetration of therapeutics, increased survival, and decreased metastasis in combination with chemotherapies; phase 1 trial with gemcitabine had 42% response rate in pancreatic cancer	Provenzano et al., 2012; Jacobetz et al., 2013; Mittapalli et al., 2013

BAPN, β-aminopropionitrile; DHB, ethyl 3,4 dihydroxybenzoate; DPEN, diphenylethylenediamine.

tumorigenicity and metastasis. Given this, HYAL inhibitors have begun to be developed. The use of sulfated hyaluronic acid compounds (normal HA is not sulfated) to inhibit HYAL1 had tumor-inhibitory effects in vitro—it blocked proliferation, motility, and invasion of prostate cancer cells; induced apoptosis; and inhibited tumor growth in animal prostate cancer models (Benitez et al., 2011).

A PEGylated human recombinant PH20 hyaluronidase (PEGPH20), which is an enzyme that degrades HA, has been developed to treat pancreatic cancer. The rationale for this is to alleviate the high pressure created by HA-enriched matrix, thus restoring blood flow and circulation to facilitate dispersion and absorption of drugs. Indeed, in pancreatic cancer preclinical trials, it did just that: PEGPH20 inhibited tumor growth, lowered IFP and increased penetration of therapeutics, and increased survival and decreased metastasis in combination with chemotherapies (Provenzano et al., 2012; Jacobetz et al., 2013). In a phase 1 clinical trial, PEGPH20 in combination with gemcitabine had a 42% response rate in stage IV metastatic pancreatic cancer (Mittapalli et al., 2013). Together, these data suggest that targeting unique ECM proteins could be fruitful in altering the mechanical properties of the tumor microenvironment, thus impinging on tumor development and progression.

The role of deregulated ECM remodeling and deposition in cancer has only recently been appreciated. The inhibitors and molecules detailed in this section (Table 2) are the first attempts in the development of cancer therapies targeting the ECM. To date very few of these inhibitors have entered into clinical trials of any kind, and the therapies tested in preclinical trials have shown mixed efficacy and a variety of off-target effects. This is, especially in the case of HA, partially due to a lack of understanding of the differential effects that various isoforms can have in different physiologic contexts. For example, small oligo HA treatment actually promoted tumor progression in some instances due to the extensive modifications that HA molecules undergo. In order for the therapies targeting HA binding, production, or degradation to work specifically and effectively, it is essential to have a clearer molecular understanding of the roles of different subtypes of HA chains in the context of specific cancer types.

Summary and Future Directions

Extensive data summarized in this review provide strong support for a critical role of mechanical properties of ECM on tumor development and metastasis. The importance of tissue stiffness in cancer has only been appreciated recently, and how tissue rigidity or stiffness regulates cancer development and metastasis at the molecular level remains largely unknown. Past decades of pioneering work in the field of mechanobiology has laid the foundation to begin formulating targeted therapies, and those discussed in this review are summarized in Fig. 1. Although this list remains relatively small, as this field continues to expand, the amount of potential therapeutic targets will as well. In addition to uncovering new players in these mechanotransduction pathways, understanding how mechanical forces exerted from the tumor ECM alters known key oncogenic and metastasis biochemical signaling pathways could repurpose existing

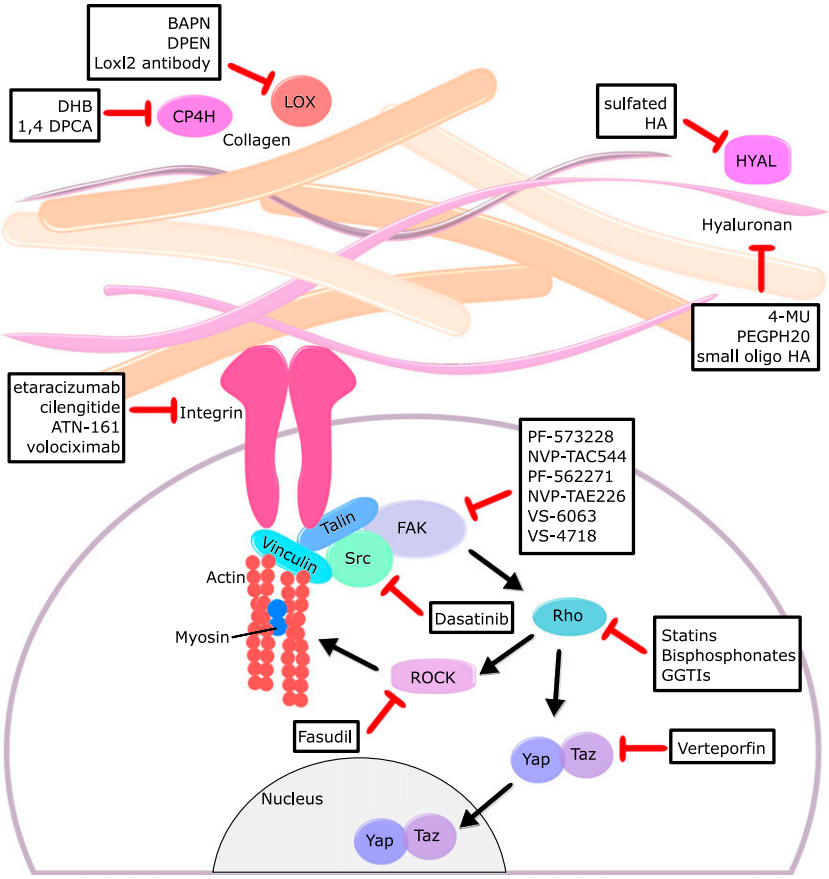

Fig. 1. A summary of various molecules implicated in mechanoregulation of cancer and the therapeutics developed against these targets. Current strategies to target mechanotransduction in cancer focus on two areas: one is to target biomechanical molecules activated by mechanotransduction, and the other one is to target the altered ECM components themselves. The first startegy has largely focused on inhibiting the well-established mechanoactivators such as integrins, FAK, and Src, and the known mechanotransducers such as RHO/ROCK. Recently, some mechanotransduction effectors such as YAP/TAZ proteins have also been experimentally targeted and show benefits in inhibiting tumor growth and metastasis. For the ECM components, most therapies are designed to inhibit the molecules responsible for creating stiffer tumor-associated matrices, including regulators of collagen synthesis such as collagen prolyl 4 hydroxylase, the LOX family of collagen crosslinking enzymes, and HA and its regulator hyaluronidase. Individual mechanoregulators are labeled in the figure, and the therapeutic agents are listed in the boxes.

therapeutics to counter the impact of mechanical forces on tumor development and progression.

Currently, most drug screens are still performed using cells cultured on conventional plastic dishes with rigidities beyond physiologic ranges. Recent efforts to incorporate physical properties of ECM into early drug-screening conditions, such as performing drug screen in three-dimensional cultures with appropriate matrix rigidities, could also improve the likelihood to identify drug candidates that will be effective in downstream preclinical animal models and subsequent clinical trials.

Acknowledgments

The authors apologize to the many researchers in this field whose work could not be cited due to space restrictions.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Majeski, Yang.

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