


THEME

Dynamic Tumor Heterogeneity and Cancer Progression

The mechanobiome: a goldmine for cancer therapeutics

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Abstract

Cancer progression is dependent on heightened mechanical adaptation, both for the cells' ability to change shape and to interact with varying mechanical environments. This type of adaptation is dependent on mechanoresponsive proteins that sense and respond to mechanical stress, as well as their regulators. Mechanoresponsive proteins are part of the mechanobiome, which is the larger network that constitutes the cell's mechanical systems that are also highly integrated with many other cellular systems, such as gene expression, metabolism, and signaling. Despite the altered expression patterns of key mechanobiome proteins across many different cancer types, pharmaceutical targeting of these proteins has been overlooked. Here, we review the biochemistry of key mechanoresponsive proteins, specifically nonmuscle myosin II, α -actinins, and filamins, as well as the partnering proteins 14-3-3 and CLP36. We also examined a wide range of data sets to assess how gene and protein expression levels of these proteins are altered across many different cancer types. Finally, we determined the potential of targeting these proteins to mitigate invasion or metastasis and suggest that the mechanobiome is a goldmine of opportunity for anticancer drug discovery and development.

α -actinin; filamin; mechanoresponse; metastasis; myosin

INTRODUCTION

Cancer is fundamentally a disease of altered mechanics. Every step—from proliferation and growth of the original tumor to dissemination and intravasation for new metastatic niches—depends on evolving internal mechanical machinery. As with healthy cells, cancer cells must be able to integrate chemical and physical signals from their external environments. But, unlike their healthy counterparts, the survival of cancer cells is contingent upon their ability to adapt to mechanically distinct microenvironments. This adaptation requires the mechanobiome, which includes the collection of proteins that are uniquely poised to respond to mechanical stresses as well as other proteins, which help set the mechanical and force-producing activities of the cell. The full mechanobiome then constitutes a large integrated network that couples these mechanical systems with a host of other cellular systems, including gene expression, cell signaling, and metabolism, among others (1, 2). These mechanoresponsive proteins, defined as those that accumulate in response to applied mechanical stresses, include the force-generating motor protein nonmuscle myosin II and specific

paralogs of the actin-crosslinking proteins α -actinin (ACTN) and filamin (1, 3) (Fig. 1, A and B). Many of these proteins have partnering proteins, such as α -actinin's CLP36 and the 14-3-3 proteins. Both CLP36 and 14-3-3s undergo significant expression changes during cancer development (Fig. 1A).

In numerous cancer types, the expression levels of mechanoresponsive protein families are often significantly altered in a highly paralog-specific manner—isoforms that are mechanoresponsive tend to have increased expression levels, whereas those that are not often show decreased or steady expression correlated with cancer progression. Changes in the expression level of even low-abundance mechanoresponsive isoforms reflects a reprogramming of cancer cells that favors increased adaptability required for efficacious growth and metastasis (4) (Fig. 1C).

Mechanoresponsive isoforms can be predicted based on their differential biochemistry, such as actin-binding affinities and myosin bipolar filament assembly dynamics (3, 5, 6). This differential biochemistry can be leveraged to develop anticancer chemical screens that are isoform specific. Although the development of most anticancer therapies relies on target inhibition, the mechanoresponsive machinery



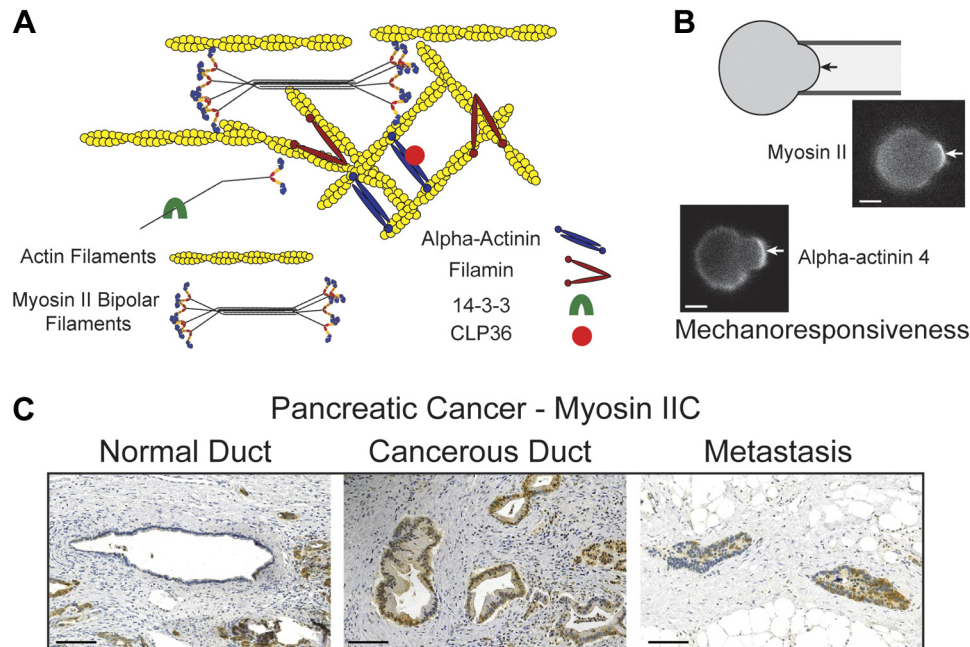


Figure 1. Mechanoresponsive proteins provide cell structure and adaptability to mechanical stresses, and their expression levels are frequently elevated in cancer progression. **A:** mechanoresponsive proteins include paralogs of nonmuscle myosin II, α -actinin, and filamin. Myosin II assembles into bipolar filaments, which are then organized into the actin meshworks and stress fibers in the cells. α -Actinins are antiparallel dimers, which organize actin filaments into bundles. Filamins on the other hand are v-shaped dimers that also crosslink actin filaments. 14-3-3 proteins can bind to myosin II tails regions where they modulate myosin II bipolar filament assembly, in addition to other biochemical functions. CLP36 binds to α -actinin and associates with actin-rich structures including stress fibers and the cell cortex. **B:** mechanoresponsiveness (defined as the ability to accumulate locally in response to applied mechanical stresses) is well revealed by micropipette aspiration (3, 4). The protein accumulates in the region of the cortex deformed by the suction pressure (arrow), increasing in intensity relative to the opposing cortex. Scale bars, 7 μ m. **C:** in pancreatic ductal adenocarcinoma, many mechanoresponsive proteins elevate in expression. As an example, myosin IIC is found at low expression levels in normal ductal epithelia. As the ductal adenocarcinoma forms, myosin IIC elevates in expression and persists in metastases (shown on the right is a metastasis found in a lymph node). Increases in the relative levels of low-expressing proteins, as compared with healthy tissue, can have dramatic effects on cancer cell behavior. Scale bars, 100 μ m. [B and C adapted from Surcel et al. (4) with permission.]

provides a target space in which activation of key components may reduce metastatic potential while protecting healthy tissue. This targeted push of the adaptive system out of its optimum (sweet spot) of activity—the position of the system that allows for maximal adaptability and ultimately, progrowth, invasion, and metastatic potential—requires a thorough understanding of the mechanobiome players. Here, we will review the mechanoresponsive protein families and what makes some paralogs mechanoresponsive and others not, their overall and relative protein concentrations in different cancer types, and some early indicators of pharmacological success in targeting of the mechanobiome for cancer therapies.

MECHANORESPONSIVE PLAYERS

Nonmuscle Myosin II

Nonmuscle myosin II (NMII) is a member of the myosin superfamily and the major active force generator on the actin cytoskeleton. Myosin II's functional unit is the bipolar thick filament (Fig. 1A). For mammalian systems, nonmuscle myosin IIs are composed of 10–30 hexameric monomers, where each monomer consists of two heavy chains, two essential light chains, and two regulatory light chains. Myosin II head domains use ATP hydrolysis to induce conformational changes that are propagated through the lever

arm, allowing the motor to pull on and generate force on the actin filament.

Mammals have three paralogs: NMIIA, NMIIIB, and NMIIIC, with unique mechanochemistry and cellular distributions. Of the three, NMIIA has the highest rate of ATP hydrolysis, pulling along actin filaments more rapidly than NMIIIB or NMIIIC (7), whereas NMIIIB has a much higher duty ratio (the amount of time the motor domain is strongly bound to the actin filament in a force-generating state) than NMIIA (8, 9). In the majority of cells studied, NMIIA and NMIIIB collectively form the largest pool of NMIIs, with NMIIIC forming the smallest pool. All three isoforms can be mechanoresponsive (3, 4). Across multiple cell types, NMIIA and NMIIIC always respond to internally and externally applied stresses, whereas NMIIIB is situationally mechanoresponsive, dependent on its assembly and regulation by the protein kinase PKC ζ (10).

In addition, each isoform has distinct roles in motility, adhesion, and other mechanically driven processes. In cancer, NMIIs play essential roles in tumor initiation, tumor formation and growth, and metastasis, all driven by their role in adhesion, mechanotransduction, motility, and contractility. During adhesion-dependent single-cell migration, for example, the localized activity of distinct NMIIs determines migratory speed and persistence, with NMIIA typically at the front promoting protrusions and adhesion maturation, and NMIIIB in the back forming the contractile rear, promoting

cellular detachment from the substrate, and impacting nuclear distortion [reviewed in (11)]. In collective cell migration, NMIs are generally considered to generate restrictive forces that help limit protrusion formation at the sides and rear of the tissue mass, rectifying Arp2/3-mediated protrusion formation toward the front of the mass and promoting collective tissue movement [e.g. (12)]. However, it should be noted that in a colorectal cancer model study, the opposite was the case whereby inhibiting upstream regulators of myosin II and myosin II itself promoted collective migration (13). In glioma, NMII is required for contractility through the brain tissue's submicrometer pores (14). Interestingly, inhibition of NMII in glioblastoma does block invasion but then leads to faster tumor growth, as myosin II is needed to inhibit pro-growth pathways, such as the ERK pathway. These two cancer examples identify a tumor suppressor role for myosin II and may underscore the "optimum/sweet spot" concept where different systems can be uniquely poised as well as highlight how the various cellular systems are integrated with one another (myosin II, signaling, growth pathways, etc.) (15). Additionally, NMIIA and NMIIIB are both found in stress fibers: in younger stress fibers, predominately transverse arcs and radial stress fibers, NMIIA is enriched, whereas in older stress fibers and longer-lived ventral ones, NMIIIB is predominant (16–18). Less is known about the lower-in-abundance NMIIIC, but all three isoforms are involved in the retrograde flow of actin (4, 19, 20).

The list of cancers with altered myosin II expression and/or regulation is extensive and includes breast, lung, prostate, bladder, pancreatic, melanoma, colorectal, ovarian, uterine, glioma, and squamous cell carcinomas (4, 14, 15, 21–27) (Fig. 2 and Table 1). Despite their ubiquitous presence, NMIs and their regulators have been the target of some anticancer drug development. For example, Rhodblock6 inhibits Rho kinase (55), thiosemicarbazone iron chelators block rho-associated protein kinase/regulatory light chain (ROCK/RLC) (56), and BDP529 inhibits myotonic dystrophy kinase-related CDC42-binding kinase (MRCK), reducing breast cancer tumor cell motility and invasion (57). In addition, 17e inhibits the myosin light-chain phosphatase, impacting prostate cancer cell growth (58), and Berberine, a RhoA/ROCK inhibitor, decreases colorectal cancer growth but also impacts cardiac and smooth muscle (59, 60). Moreover, Fasudil, also a ROCK inhibitor, inhibited cellular contractility, decreasing pancreatic tumor stiffness and making the cancer cells more sensitive to cytotoxic drugs, gemcitabine and Abraxane, a standard of care for patients with pancreatic cancer (61). As a result, Fasudil increased cell death and reduced the metastatic potential of pancreatic cancer cells in mouse models. This example highlights the potential of synergizing myosin II regulator modulation with other treatment strategies.

Most of the aforementioned small molecules show no isoform specificity. However, NMII distribution across a wide variety of cancers reflects differential expression patterns of NMIIA, IIB, and IIC, suggesting that isoform-specific targeting compounds may be a better approach. One such possibility is 4-hydroxyacetophenone (4-HAP). Identified in a screen for mechanical modulators, 4-HAP specifically activates NMIIIB and NMIIIC but not NMIIA. In pancreatic cancer, the mechanoresponsive isoforms NMIIA and NMIIIC expression increases, whereas the conditionally mechanoresponsive

NMIIIB decreases [NMIIIB is nonmechanoresponsive in pancreatic cancer cells (4)]. In both pancreatic and colorectal cancer models, treatment with 4-HAP leads to decreased dissemination in *in vitro* models and decreased metastatic tumor burden in hemisplenectomized mice (4, 62). In addition, 4-HAP impacts the mechanics of circulating tumor cells from breast cancer (63).

What this cluster of studies suggest is that targeting specific isoforms in the mechanobiome impacts cancer cell behavior and has potential therapeutic benefit. Understanding the characteristics that underlie the biochemical differences among mechano- and nonmechanoresponsive isoforms, can lead to better approaches for drug discovery platforms.

Actin Crosslinkers

α -Actinins.

α -Actinins are a family of cross-linking proteins that includes four paralogs. The muscle-associated actinins are α -actinin 2 and 3 and are encoded by ACTN2 and 3, whereas nonmuscle isoforms are encoded by ACTN1 and 4, respectively. The muscle isoforms are found in skeletal and smooth muscle and organize actin filaments into sarcomeric structures, which constitute the minimal contractile unit. The nonmuscle paralogs organize actin filaments into the networks that contribute to nonmuscle cell structure, migration, and adhesion (Fig. 1A). ACTN4 has been linked to metastasis in cancer through alterations in cellular features and cellular misbehavior (64–67).

Based upon stopped flow kinetic measurements and actin filament cosedimentation assays using single actin-binding domains, mammalian α -actinin 1 and α -actinin 4 have a ~90-fold differential in binding affinities for actin, with K_{ds} of 0.36 μ M and 32 μ M, respectively (68, 69). This major difference in binding affinity coupled with the force-dependent bond formation (catch-slip bond) observed for these actin crosslinking proteins was sufficient to predict which of the α -actinin paralogs would accumulate in response to mechanical stress (3). As predicted and confirmed experimentally, α -actinin 4 senses mechanical stress on the actin cytoskeleton, resulting in accumulation to sites of external stress whereas α -actinin 1 does not. Accumulation depends on catch-slip bond formation and an optimal actin-binding affinity, impacting cells that need to be highly adaptive like cancer cells.

Amazingly, these two ACTNs also differ in how their expression changes as a normal pancreatic ductal epithelia cell transforms into a ductal adenocarcinoma (4). ACTN1 is already abundant in normal pancreatic ductal epithelial cells and rises somewhat in the cancer cells and across the cancer stroma.

Meanwhile, ACTN4 is poorly expressed in healthy pancreatic ductal epithelial cells but then dramatically increases only in the ductal adenocarcinoma.

Expression level changes of ACTN genes and their proteins are observed in multiple types of cancers (Fig. 2 and Table 2). For example, ACTN4 has a decreased expression level in endometrial, neuroblastoma, and prostate cancer, relative to normal tissue. In contrast, the expression levels of ACTN4 are increased in breast and pancreatic cancer, among others, as compared with the relevant healthy tissue (Table 2). What might these expression changes mean? First, the

reduced expression of ACTN4 impairs fibroblasts in cell migration, spreading adhesion and proliferation, suggesting that α -actinin 4 is necessary for normal cell morphology and motility (111). In contrast, the overexpression of ACTN4 promotes breast cancer tumorigenesis through enhanced cell motility (112). Gene expression profiles from patient primary lung cancer, adjacent benign tissue, and metastatic brain tumor show that ACTN4 is elevated in the metastatic brain tumor and leads to lung cancer metastasis to the brain (113). In colorectal cancer cells, α -actinin 4 provokes immature focal adhesions, which leads to cell motility and invasion, whereas α -actinin 1 does not (114). Additionally, patients with an increase in the copy number of ACTN4 have metastatic phenotypes leading to lower prognosis, like salivary gland

carcinoma (109). Thus, ACTN4 appears to play a role in cancer progression and metastasis whereas ACTN1 appears not to.

Filamins.

The filamins are a family of actin-binding proteins that can associate with F-actin filaments in a solution to result in a dense gel meshwork (115). In humans, the filamin family includes three isoforms: filamin A (FLNA), filamin B (FLNB), and filamin C (FLNC) (116). FLNA and FLNB are widely expressed in various human tissues (117), whereas FLNC is primarily expressed in cardiac tissue and skeletal muscle (118, 119). Collectively, filamins are found in the cell cortex, the F-actin-rich region underlying the plasma membranes (116, 120–122) (Fig. 1A). In mammalian cells, nonmuscle

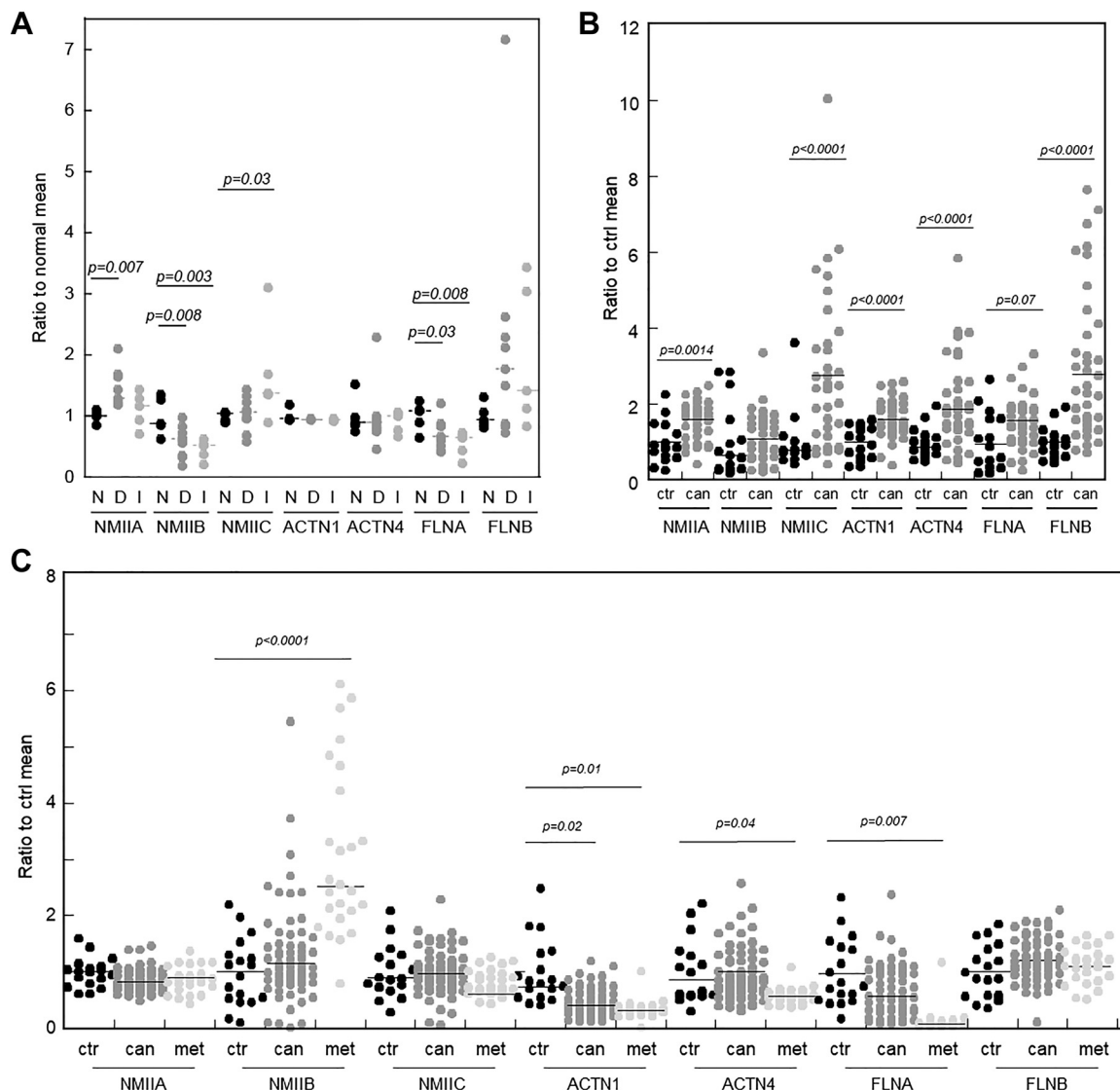


Figure 2. Pancreatic and prostate, but not breast, cancers have one or more mechanoresponsive protein-encoding genes upregulated as compared with healthy tissue. These data were collected from Gene Expression Omnibus (GEO) Repository, which assesses transcript levels. **A:** in human patient-derived ductal carcinoma in situ (DCIS; D), and invasive ductal carcinoma (IDC; I) breast cancer vs. normal tissue (N), some mechanoresponsive proteins have increased gene expression while nonmechanoresponsive paralog-encoding genes remain unchanged or are decreased (53) (BioProject: PRJNA126373). **B:** in pancreatic cancer, several mechanoresponsive protein transcript levels are increased in patient pancreatic cancer tissue relative to normal tissue (54) (BioProject: PRJNA116073). **C:** metastatic prostate cancer samples from patients with androgen-ablation-resistant metastatic tumors as well as primary tumor samples relative to normal prostate tissue revealed elevation of NMIIIB gene expression (52) (BioProject: PRJNA104173, 104175, 104177, 104179). NMII, nonmuscle myosin II.

Table 1. Expression trends of nonmuscle myosin II in various cancers

Cancer Type	Myosin IIA			Myosin IIB			Myosin IIC		
	Protein Cell Lines	Protein Human Tissue	mRNA Human Tissue or Cell Lines	Protein Cell Lines	Protein Human Tissue	mRNA Human Tissue	Protein Cell Lines	Protein Human Tissue	mRNA Human Tissue or Cell Lines
Acute myeloid leukemia Bladder Breast	Decreased (31) Increased (22)	Increased (27)	Increased (28) Increased (27) Increased (31) Increased in cell lines (32)	Decreased (31)	Increased (29) No change (22)		Increased (31)	Increased (30)	Increased in cell lines (32)
		Increased (33)	Increased (34)						
		Increased (35) Increased (36–38)	Increased (36,37)						
	Increased (14)			Variable (14) Increased (39) Decreased (40)	Variable (14)	Increased (39)	Variable (14)	Variable (14)	
		Decreased (41)							
Head and neck, including tongue and larynx Liver Lung Melanoma	Decreased (31)	Increased, Spitz tumors (43)	Increased (42)	Decreased (31) Increased (44)	Increased (44)	No change (31)	Increased (31)		
	Increased (45) Increased (4)	Increased (46) Increased (4, 47)		Increased (45) Decreased (4)	Decreased (4)		Increased (4)	Increased (4)	
		Increased (48)			Favorable prog. indicator (49)	MYH10-RET gene fusion (50)		Favorable prog. indicator (49)	
Prostate Renal									
Thyroid Salivary gland Squamous cell carcinoma Stomach	Increased (51)		Decreased (26)						
Urothelial					Unfavorable prog. indicator (49)			Favorable prog. indicator (49)	

Prog., prognosis.

Table 2. Expression trends of α -actinins in various cancers

Cancer Type	α -Actinin 1			α -Actinin 4		
	Protein Cell Lines	Protein Human Tissue	mRNA Human Tissue	Protein Cell Lines	Protein Human Tissue	mRNA Human Tissue
Acute myeloid leukemia		Variable (70)				
Bladder				Increased (71)	Increased (71, 72)	Increased (71)
Breast		Increased (73)		Increased (32)	Distribution pattern correlates w/ prognosis (65); Increased (74)	Increased (75, 76)
Cervical					Increased (77,78)	
Colorectal		Unfavorable prog. indicator (49)			Increased (79)	
Endometrial					Decreased (80, 81)	
Esophageal					Increased (82, 83)	
Gastric					Increased (84)	Increased (84)
Glioma		Increased (85)			Increased (85, 86)	
Head and neck, including tongue and larynx		Unfavorable prog. indicator (49)				Increased (87, 88)
Liver						
Lung		Unfavorable prog. indicator (49)		Increased (89)	Increased (90); Unfavorable prog. indicator (49)	Increased (91)
Melanoma				Increased (92)	Increased (93)	
Neuroblastomas				Decreased (94)		
Ovarian					Increased (95–98)	Increased (95, 97, 98)
Pancreatic		No change (4)			Increased (4, 99–101); Unfavorable prog. indicator (49)	Increased (79)
Prostate		Decreased (102)			Decreased (104, 105)	
Renal		Increased (103)			Favorable prog. indicator (49)	
Thyroid		Unfavorable prog. indicator (49)			Increased (107)	Increased (108)
Salivary gland		Increased (106)				Increased (109)
Squamous cell carcinoma				Increased (110)	Increased (110)	
Stomach						
Urothelial		Unfavorable prog. indicator (49)				

Prog., prognosis.

filamin A and filamin B form dimers, crosslink actin filaments, and can link the actin filaments to integral membrane proteins, helping to couple the cortical actin meshwork to the plasma membrane (116).

Similar to α -actinins, filamin isoforms also have distinct actin-binding affinities though the range is not as broad [Filamin B, $K_d = 7 \mu\text{M}$ (123); Filamin A, $K_d = 17 \mu\text{M}$ (124)]. Filamin B is highly mechanoresponsive, whereas filamin A is much less so (3, 4). Filamin B and A have a flipped relationship between binding affinity and mechanoresponsiveness as compared with the α -actinins where the lower affinity paralog α -actinin 4 is the more mechanoresponsive isoform. This difference may reflect that filamins are more subtly tuned and also have a cooperativity component that contributes to their mechanism of mechanoresponsiveness (3, 5). Consistent with this, filamin A is necessary for the active stiffening of melanoma cells that are plated on collagen, but in response to large external forces, filamin A is not required for passive stiffening (125). In addition to the mechanoresponsiveness, other factors also help modulate filamin-actin-binding affinity. For example, filamins bind tropomyosin-

bound F-actin with increased affinity ($K_d = 0.13\text{--}3.2 \mu\text{M}$) (126). The interaction between filamin and F-actin can also be regulated by phosphorylation (127), inositol phospholipids (128), and Ca^{2+} -calmodulin (124).

In vitro, increasing the ratio of filamin to actin leads to tighter networks (129). In cells, increasing the filamin concentrations during cancer progression could shift the type of actin filament organization with differing effects on cell behavior, depending on the starting point. As a starting reference point, in melanoma cells, the ratio of filamin to actin has been measured to be around 1:80–140 (130). In these cells, increasing FLNA enhances invasive ability of human melanoma cells (131). In contrast, in many scenarios, FLNA inhibits cell growth and metastasis (132). For example, recent research found that the expression of the FLNA gene can inhibit the malignancy of prostate adenocarcinoma (133) and breast cancer cell migration and invasion (23, 134) and can regulate colorectal cancer cell growth and migration (135). Additionally, FLNB is upregulated in pancreatic cancerous ducts, whereas FLNA is upregulated across the pancreatic tissue and stroma (4). Among other human cancers (Table

3), B cell childhood acute lymphoblastic leukemia and Pro-B acute lymphoblastic leukemia have an increase of FLNB mRNA expression when compared with normal tissues (136).

Overall, highly mechanoresponsive ACTN4 and FLNB paralogs are consistently associated with greater invasion and metastasis, whereas the non- or less-responsive paralogs are generally associated with less invasive and metastatic potential across many cancer types. Metastatic cells need to remodel their network to undergo shape change rapidly as they migrate through an ever-changing microenvironment. A shift in binding affinity in response to mechanical stress is a major driver for the mechanoresponsiveness and upregulation of ACTN4 and FLNB. Chemical screens could be developed that leverage isoform-specific biochemistry toward therapeutic treatments. Although inhibition is one possible strategy, compounds that alter cancer cells' mechanoresponsiveness by shifting the force sensitivity of the crosslinker's actin-binding activity, making α -actinin 4 or filamin B more α -actinin 1- or filamin A-like, respectively, may provide an alternate therapeutic strategy.

Other Interacting Players

Due to the isoform-specific activity of mechanoresponsive proteins, some of their cofactors, such as 14-3-3 and CLP36, may be of interest to help mediate invasive potential.

14-3-3

The human 14-3-3 family comprises seven isoforms (β , γ , ϵ , η , σ , θ , and ζ), each expressed by a different gene (146). 14-3-3s generate interest because of their roles in signal transduction pathways that control cell cycle checkpoints (147), MAP kinase activation (148), apoptosis (149), and DNA damage repair (150). 14-3-3s have been studied extensively as a drug target by either stabilizing or inhibiting protein-protein interactions. For example, 14-3-3-targeting drugs include fusicocanes, which stabilize 14-3-3 binary structures (151), pyrrolidone1 and epibestatin, which were identified in a high-throughput screen for 14-3-3 stabilizers (152), and phosphonate-type inhibitors, PPI inhibitor 7 (153), among many more summarized in (154).

14-3-3 was originally identified as an interactor of myosin II through a combination of genetics, cellular biophysics, and proteomics assays in *Dictyostelium discoideum* (155). For the mammalian counterparts, based on a suite of in vitro assays, 14-3-3 paralogs, especially 14-3-3 σ , inhibit myosin II filament assembly, whereas 14-3-3 ζ had no effect on myosin IIB or IIC but promotes assembly of myosin IIA (156) (Fig. 1A). The mammalian 14-3-3-myosin II interaction has a relatively high affinity with an apparent K_d of 380 nM (156).

The 14-3-3 protein family plays a major role in cancer, and the majority of the isoforms are upregulated in a variety of

Table 3. Expression trends of filamins in various cancers

Cancer Type	Filamin A			Filamin B		
	Protein Cell Lines	Protein Human Tissue	mRNA Human Tissue	Protein Cell Lines	Protein Human Tissue	mRNA Human Tissue
Acute myeloid leukemia						
Bladder						Increased (136)
Breast		Increased (137, 138)	Increased (137)			Increased (136)
Cervical		Increased (139, 140)				
Colorectal		Increased (138, 141); Unfavorable prog. indicator (49)				Decreased (136)
Endometrial						
Esophageal						Increased (136)
Gastric		Decreased (142)				Decreased (136)
Glioma	Increased (143)					
Head and neck, including tongue and larynx						Increased (136)
Liver		Increased (138)				
Lung		Increased (138, 144)				
Melanoma						Decreased (136)
Neuroblastomas						
Ovarian		Increased (138)				Increased (136)
Pancreatic		Increased (138)			Increased (4) Unfavorable Prog Indicator (49)	Decreased (136)
Prostate		Increased (138, 145)				Increased (136)
Renal		Unfavorable prog. indicator (49)			Favorable prog. indicator (49)	Decreased (136)
Thyroid						
Salivary gland						
Squamous cell carcinoma						Increased (136)
Stomach						
Urothelial		Unfavorable prog. indicator (49)				

Prog., prognosis.

disease states [reviewed in (157)] (Table 4). For example, 14-3-3 σ is strongly upregulated in colorectal cancer cells (147) and functions as a tumor suppressor in breast (160) and gastric cancer (222). Additionally, 14-3-3 σ is upregulated in lung cancer (223), head and neck squamous cell carcinomas (194), and chemoresistant pancreatic adenocarcinoma cells (224). In astrocytoma, 14-3-3 β seems to have distinct tissue localization and increased protein expression (225). In several cancer types, the seven 14-3-3 isoforms exhibit different ratios of expression. For example, all the isoforms have increased cytoplasmic levels in vulvar carcinoma, with the exception of 14-3-3 θ , which has decreased expression (226). Amazingly, expression levels of 14-3-3 ϵ , ζ , and θ increased with the increase of pathological grade of meningioma, whereas the 14-3-3 η , β , γ , and σ isoforms were reduced in expression (227). A group reported that tamoxifen treatment appears to induce 14-3-3 ζ expression in breast cancer cells (228). A thorough understanding of isoform-specific function is needed, as proteins in the mechanobiome are interconnected and affect each other's expression and/or activity. For example, overexpression of 14-3-3 ϵ in a human gastric cancer cell line resulted in an increase in the total cellular level of filamin A and an increase in the subcellular localization of filamin A in the cytoplasm (229).

CLP36/PDLIM1

CLP36 (also known as CLIM1 or Elfin) is a 38-kD protein that has an N-terminal PDZ domain and a C-terminal LIM domain. Due to the association between actin and α -actinins in cancer, CLP36 becomes of interest here because of its interactions with α -actinins (Fig. 1A). CLP36 associates with actin filaments during cell shape changes, migration, and during the contraction of endothelial cells. CLP36 is also expressed in most nonmuscle tissues though in some, such as the pancreas, expression can be very low (230). However, in situ hybridization analysis of mouse tissues revealed that CLP36 can be highly expressed, and when present, it localizes to actin stress fibers through the PDZ domain and interacts with α -actinin 1 and α -actinin 4 (231). During cell shape change events, including cell spreading, migration, and contraction, CLP36 associates with actin filaments and stress fibers (231, 232). ACTN4 is highly expressed in the colon, and again in this context, CLP36 interacts with α -actinin 4, forming stable α -actinin 4-CLP36 complexes, which extends throughout the actin stress fibers (231). CLP36 also showed significant changes in levels in breast cancer as compared with ovarian cancer patient plasma samples (233). Thus, the mechanoresponsive proteins and interacting players have potential for early detection of cancers. For example, patients with breast cancer can have increased tumor-associated autoantibodies to CLP36 (234). Understanding the protein interactions between CLP36 and α -actinin 4 may provide additional insight into how to modify their activities toward a therapeutic end.

Conclusions and Vision

The mechanoresponsive machinery plays a central role in many cell shape change events and is also heavily associated with cancer progression and metastasis. Further, many of

these proteins have expression levels that can be altered in different ways in different cancer types that likely then lead to the altered cell mechanics and mechanoresponsiveness associated with cancer progression (4, 69, 113, 134, 235) (Fig. 2 and Tables 1–4). Despite their differential cancer expression levels, the mechanobiome proteins have been largely overlooked in drug development and trials. This oversight is caused by four primary assertions.

The first is that the classic view of these proteins often prescribes a singular major role in the cell, without taking into account the diversity of functions that can be attained by varying ratios between the paralogs. For example, non-muscle myosin II's textbook definition is that of contractility required for cytokinesis and motility. However, myosin II's functions are much more extensive and include roles in mechanosensation, elasticity and viscoelasticity, cortical tension and fluidity, the modulation of cell adhesion to substrates as well as other cells, the integration between signaling and mechanical inputs, and the impact on overall cell mechanics on many other cell functions (3, 236–238). It is the interplay between the three myosin isoforms that skews cell behavior in disease states (4, 15); understanding how the isoforms participate fully in all NMII functions will shed light on how changes in their expression yield transformative cancer cells. Similarly, the preponderance of research on 14-3-3 in the cancer field has focused on its role in processes such as the DNA damage response [e.g., (161)], with less focus on its interactions with cytoskeletal components (156, 239). With seven isoforms that exhibit some overlapping function and differential expression in multiple cancer types, fully defining their function will allow for better targeting of individual 14-3-3 isoforms in cancer.

The second is that ubiquitous expression of mechanoresponsive proteins across multiple cell types has led to the assumption that their targeting would be toxic to human patients. This is despite the fact that proteins Kras, Rho, and Aurora kinase, which are also abundantly expressed, are the target of multiple drug trials for cancer [e.g., reviewed in (240, 241)]. In addition, the families of proteins that are mechanoresponsive are often treated as an aggregate of all of their isoforms or of the most abundantly expressed isoform. This is most starkly seen with the myosins, where the preponderance of research on nonmuscle myosin IIs is focused on IIA and IIB, with little consideration to IIC due to its low abundance compared with IIA and IIB. However, despite its lower amounts (18 nM vs. 565 nM of IIA in pancreatic ductal adenocarcinoma cancer cells), myosin IIC helps facilitate actin organization and retrograde flow, working in concert with myosin IIA to increase dissemination and metastasis (4, 62). These data are just one example of how low abundance proteins that are often disregarded in large data mining can actually be viable candidates for drug targeting.

Although the abundance of proteins needs to be reconsidered for defining the importance of a given protein for cancer progression, it is also worth noting that mechanoresponsiveness, as well as nearly all cellular processes, can also be regulated by posttranslational modifications. In one key example, NMIIIB has exquisite cell-type-specific and even cell-cycle-stage-specific mechanoresponsiveness (10). Myosin II heavy-chain phosphorylation, carried out by PKC ζ , mediates this differential effect in mechanoresponsiveness. No doubt, this

Table 4. Expression trends of 14-3-3 in various cancers

Cancer Type	14-3-3		
	Protein Cell Lines	Protein Human Tissue	mRNA Human Tissue
Acute myeloid leukemia			
Bladder		σ: Decreased (158); Increased (159)	σ: Decreased (158)
Breast	β: No change (160) σ: Decreased (160, 161) ζ: No change (160)	ε: Increased (162, 163) θ: Increased (164, 165) σ: Decreased (160) ζ: Increased (166) β: Unfavorable Prog Indicator (49)	ε: Increased (167) σ: Decreased (161)
Cervical	β: Decreased (168) ε: Decreased (168)	σ: Increased (169) γ: Unfavorable Prog Indicator (49)	σ: Increased (169)
Colorectal	β: Increased (170) σ: Decreased (171)	β: Increased (172) ε: Decreased (173)	β: Increased (174) η: Decreased (175) σ: Decreased (175) ζ: Decreased (175)
Endometrial		σ: Decreased (176) β: Unfavorable prog. indicator (49) ε: Favorable prog. indicator (49) θ: Unfavorable prog. indicator (49) ζ: Unfavorable prog. indicator (49)	
Esophageal	σ: Increased (177) ζ: Increased (178)	σ: Decreased (177) ζ: Increased (178)	ζ: Increased (178)
Gastric	ζ: Increased (179)	β: Increased (180,181) ε: Increased (182) σ: Increased (183,184) ζ: Increased (179)	β: Increased (185) ε: Increased (185) η: Increased (185) γ: Increased (185) θ: Increased (185) ζ: Increased (185) β: Increased (188) ε: Increased (188) η: Increased (188) γ: No change (188); Decreased (190) θ: No change (188) ζ: Decreased (188) σ: Increased (194) ζ: Increased (195)
Glioma	β: Increased (186)	β: Increased (187–189) ε: No change (188)η: Increased (188) γ: No change (188); Increased (187) θ: No change (188) ζ: Decreased (188); Increased (187)	
Head and neck,including tongue and larynx	β: Increased (191) ε: Decreased (192)	σ: Increased (193) ζ: Increased (193) β: Unfavorable prog. indicator (49) β: Increased (197) ε: Increased (196, 198)σ: Decreased (199) ζ: Increased (200) β: Unfavorable prog. indicator (49) η: Unfavorable prog. indicator (49) θ: Unfavorable prog. indicator (49) ζ: Unfavorable prog. indicator (49)	
Liver	ε: Increased (196) σ: Decreased, gene (171)	β: Increased (201) ε: Increased (201) γ: Increased (204, 205) θ: Increased (201) σ: No change NSCLC (206); Increased in NSCLC (207); Decreased in SCLC (206) ; Increased (201) ζ: Increased (201) β: Unfavorable prog. indicator (49) γ: Unfavorable prog. indicator (49) ζ: Unfavorable prog. indicator (49)	
Lung Melanoma Neuroblastomas Ovarian	β: Increased (201) ε: Increased (201) γ: Increased (202); No change (201) θ: Increased (201, 203) σ: Increased (201) ζ: Increased (201)	β: Increased (201) ε: Increased (201) γ: Increased (204, 205) θ: Increased (201) σ: No change NSCLC (206); Increased in NSCLC (207); Decreased in SCLC (206) ; Increased (201) ζ: Increased (201) β: Unfavorable prog. indicator (49) γ: Unfavorable prog. indicator (49) ζ: Unfavorable prog. indicator (49)	γ: Increased NSCLC (205) σ: Increased NSCLC (207)
Pancreatic	σ: Decreased (208); Increased (209) ζ: Increased (210) σ: Variable depending on cell line (211)	σ: Decreased (208)σ: Increased (209) ζ: Increased (210) σ: Increased (212) ζ: Increased (212,213) γ: Unfavorable prog. indicator (49) ζ: Unfavorable prog. indicator (49)	σ: Increased (211) ζ: Increased (212)
Prostate	β: Increased (214) σ: Decreased (215)	ε: Increased (216) θ: Increased (216) σ: Decreased (176, 217) β: No change (218) ε: Increased (218)	ε: Increased (216) σ: Decreased (215) θ: Increased (216) β: Increased (218) ε: Increased (218)
Renal			

Continued

Table 4.— Continued

Cancer Type	14-3-3		
	Protein Cell Lines	Protein Human Tissue	mRNA Human Tissue
Thyroid Salivary gland Squamous cell carcinoma Stomach Urothelial		η : No change (218) γ : No change (218) θ : No change (218) ζ : No change (218) β : Favorable prog. indicator (49) γ : Unfavorable prog. indicator (49) ζ : Unfavorable prog. Indicator (49) β : Increased (219)	η : Increased (218) γ : No change (218) θ : Increased (218) ζ : No change (218)
	η : Increased (220)		
			ζ : Increased (221)

NSCLC, non-small cell lung cancer; Prog., prognosis.

example highlights the importance of considering the specific context of any disease, which will likely influence the best strategy for targeting the disease.

Third, and perhaps most important, proteins that are upregulated in cancers are often pursued for pharmacological inhibition. Knocking down or inhibiting mechanoresponsive proteins in cancerous systems can actually yield more disseminative behavior and animals with higher metastatic load (4, 15, 26, 242). Instead of relegating mechanoresponsive proteins as untargetable, data from several studies suggest that the pharmaceutical paradigm needs to be shifted away from inhibition alone. Mechanical adaptability exists on a continuum with cells occupying a sweet spot (optimum) between adaptability and stability for a given tissue environment. Cancer cells seek to favor all types of adaptability but have evolved to have multiple routes to ensure survival, such that inhibition alone is insufficient to stop cancerous cellular behavior, in some cases even increasing disseminative behaviors.

Instead, it is a viable strategy to develop small molecules that specifically activate mechanoresponsive proteins, pushing them into a regime where they act in a manner that is more stable than their nonmechanoresponsive sister paralogs. One way to generate this hyperactivation is to increase the binding affinity of the proteins for cytoskeletal binding partners (such as the actin filaments themselves), preventing their disassembly and reducing further diseased morphologies (Fig. 3). In fact, such a strategy has been successfully used with omecamtiv mecarbil, a selective activator of cardiac myosin currently in *phase 3* clinical trials for hypertrophic cardiomyopathies (243). In two different studies, 4-hydroxyacetophenone (4-HAP), which activates specifically myosin IIC in pancreatic ductal adenocarcinoma and colorectal cancer models by locking it onto actin filaments, shows promise that skewing the activation/inhibition curve toward activation can curb cancer behaviors in mouse models (4, 62). Looking to mechanoresponsive proteins as

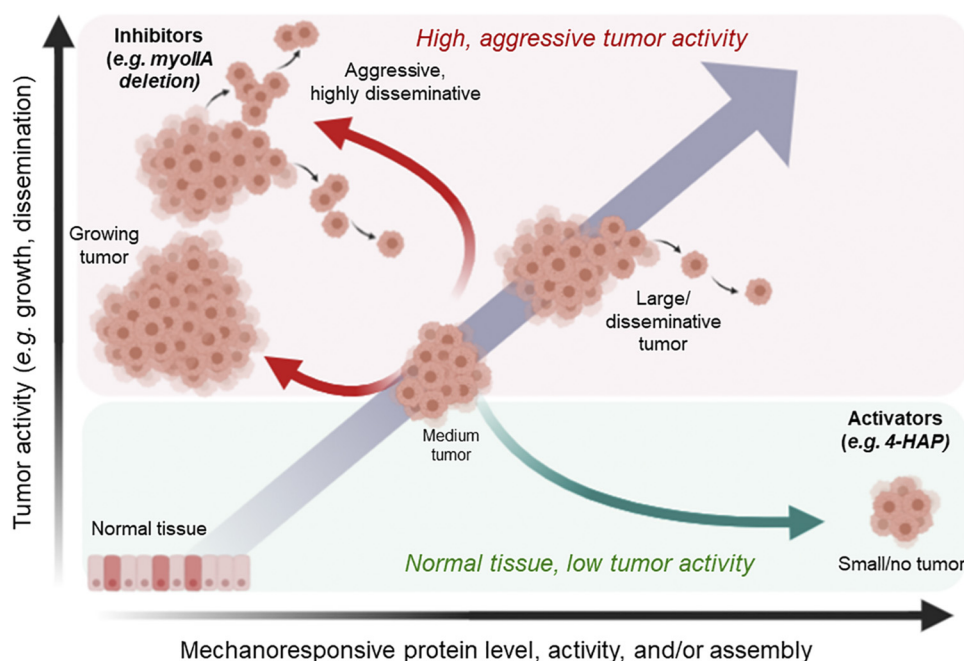


Figure 3. Shifting the activation curve of mechanoresponsive proteins and their partners is a viable strategy for developing cancer therapeutics. Cancer progression and tumor formation is marked by altered expression in the mechanoresponsive proteins, α -actinin, filamin, and nonmuscle myosin II (NMII), as well as partnering proteins such as 14-3-3 and CLP36. Left unchecked, the altered in gene expression correlate with increased cell activity, specifically metastasis. Because mechanoresponsive proteins such as NMIs also have tumor-suppressive roles as they inhibit pathways such as the ERK pathway, pharmacological inhibition can lead to enhanced tumor growth. In contrast, activators can lock in a cell, leading to anticancer behaviors. One such example is the activator 4-HAP, which increases nonmuscle myosin IIB and IIC assembly, leading to increased cortical tension and reduced tumor metastatic activity. Figure created with BioRender.com. 4-HAP, 4-hydroxyacetophenone.

targetable drug spaces (Fig. 3) can do much to change the cancer fighting landscape.

One final hurdle is that development of antimetastatic cancer treatments typically depends on measurement of primary tumor size, looking for tumor size reduction. The logic is that imaging the primary tumor is easier because of the tumor's larger mass and that tumor reduction can be a faster, suitable surrogate readout for long-term benefit of the drug for patients. However, this hurdle makes it more tempting to overlook the real potential of antimetastatic therapeutic strategies (244). Ironically, at least two traditional anticancer drugs, the DNA-damaging cisplatin and the microtubule stabilizer docetaxel, also modulate the cancer cell's mechanical properties and help reduce invasiveness (245). Perhaps improved strategies that more precisely leverage underappreciated mechanoresponsive proteins can help promote the fortitude necessary to develop new drugs that target cancer metastasis. It is also possible that the upregulation of mechanoresponsive proteins could offer a molecular program for early detection of metastases. α -Actinin 4 may already provide such an opportunity for cervical cancer (246).

Collectively, the mechanobiome, particularly the mechanoresponsive proteins and their networks, offer enormous opportunity for cancer intervention. Strategically targeting this machinery may allow for the inhibition of a wider range of cancer types at their most lethal impact point—formation of metastases—while minimizing toxicity effects for patients.

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DISCLOSURES

D. N. Robinson is currently exploring formation of a startup company named Amoibe Discovery. It is still in exploration phase. A patent has been granted on the use of 4-HAP to treat disease, and A. Surcel and D. N. Robinson are inventors on this patent. E. Parajon does not have any conflicts of interest, financial or otherwise, to disclose.

AUTHOR CONTRIBUTIONS

E.P., A.S., and D.N.R. prepared figures; E.P., A.S., and D.N.R. drafted manuscript; E.P., A.S., and D.N.R. edited and revised manuscript; E.P., A.S., and D.N.R. approved final version of manuscript.

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