# Soft Matter



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# REVIEW

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# Towards an integrative understanding of cancer mechanobiology: calcium, YAP, and microRNA under biophysical forces

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An increasing number of studies have demonstrated the significant roles of the interplay between microenvironmental mechanics in tissues and biochemical-genetic activities in resident tumor cells at different stages of tumor progression. Mediated by molecular mechano-sensors or -transducers, biomechanical cues in tissue microenvironments are transmitted into the tumor cells and regulate biochemical responses and gene expression through mechanotransduction processes. However, the molecular interplay between the mechanotransduction processes and intracellular biochemical signaling pathways remains elusive. This paper reviews the recent advances in understanding the crosstalk between biomechanical cues and three critical biochemical effectors during tumor progression: calcium ions (Ca<sup>2+</sup>), yes-associated protein (YAP), and microRNAs (miRNAs). We address the molecular mechanisms underpinning the interplay between the mechanotransduction pathways and each of the three effectors. Furthermore, we discuss the functional interactions among the three effectors in the context of soft matter and mechanobiology. We conclude by proposing future directions on studying the tumor mechanobiology that can employ Ca<sup>2+</sup>, YAP, and miRNAs as novel strategies for cancer mechanotheraputics. This framework has the potential to bring insights into the development of novel next-generation cancer therapies to suppress and treat tumors.

# 1. Introduction

Cancer is the second leading cause of human death worldwide, accounting for 10 million deaths annually.<sup>1</sup> 90% of cancer deaths are the consequence of metastasis, the process of invasive tumor cells spreading from primary solid tumors to distant organs.<sup>2–6</sup> The molecular mechanisms and parameters within primary tumors that regulate tumor progression and promote metastasis, however, are poorly understood. Such a knowledge gap in the understanding of tumor progression and prediction of metastasis onset is of serious concern. Increasingly studies from multiple disciplines, such as soft matter biophysics

and mechanobiology, have begun to demonstrate the significant influences of biophysical microenvironments and signaling on tumor initiation, progression, and metastasis (Fig. 1).<sup>7–18</sup> These influences are realized *via* mechanochemical transduction or mechanotransduction—the process in which cells sense and transduce extracellular biophysical stimuli into intracellular biochemical signals that elicit coherent biochemical responses and gene expression.<sup>14–16,19–29</sup> Advances in the understanding of mechanotransduction have led to the design and development of new classes of pharmaceuticals, drug testing and delivery systems, wearable therapeutic devices, and engineered tissues that leverage biomechanics or target mechanobiology pathways to enable innovative combinatorial therapeutics.<sup>30–39</sup>

In this review, we focus on reporting the interplay between biomechanical signals and three important biochemical effectors during tumor progression: (1) calcium ions ( $Ca^{2+}$ ), (2) yesassociated protein (YAP), and (3) microRNAs (miRNAs). Two other recent reviews discuss other types of biochemical effectors involved in cancer mechanobiology.<sup>7,31</sup> During cancer progression,  $Ca^{2+}$  and YAP serve as critical signaling messengers to regulate gene-expression and cell functions, which are simultaneously modulated by miRNAs. However, how  $Ca^{2+}$ , YAP, and miRNA interact with each other to influence the

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**Fig. 1** Overarching scientific framework of cancer mechanobiology. Biophysical signals from extracellular microenvironments transmit into cells through the mechanotransduction processes, and induce changes in intracellular biomechanics, biochemistry, biophysics, and genetics to impact healthy cell physiology and cancer cell pathobiology. Understanding of the underlying molecular mechanisms in cancer mechanobiology contributes to the design and development of novel cancer therapies. Representative mechano-regulated calcium and YAP signaling pathways are shown in the cell (center; the black scale bar represents 5 μm length).

activities of cancer cells remains incompletely understood. Further, the functional interplay between mechanical signals and these three effectors, as well as the underpinning molecular mechanisms, are still elusive.

The goal of this review is to synergistically report (1) the recent advances in the understanding of these three effectors and their crosstalk with mechanotransduction pathways during cancer development, and (2) the mechanistic insights from soft matter and mechanobiology perspectives into the orchestrated functions of these three effectors. First, we introduce and discuss the current findings that demonstrate how biophysical forces induce calcium signaling in cancer cells and the identified molecular mechanisms (Section 3). Second, we address the functional responses of YAP to biophysical signals and the roles of cell cytoskeleton and nucleus in YAP mechanosensing (Section 4). Third, we discuss the functional interactions of miRNAs with Ca<sup>2+</sup> and YAP, and the crosstalk between miRNAs

and mechanical signaling in cancer (Section 5). We conclude by proposing promising future directions on the study of tumor mechanobiology using  $Ca^{2+}$ , YAP, and miRNAs as potential targets, as well as novel strategies for cancer mechanotherapeutics (Section 6).

### 2. Mechanotransduction in cancer

All living cells and tissues in the human body experience biophysical forces from their micro- and macro-environments, including but not limited to tension, shear stress, compression, and fluid pressure.<sup>40–43</sup> At the same time, cells actively generate and apply endogenous forces to their surroundings.<sup>31,41,44</sup> The biophysical cues are sensed and transduced into intracellular biochemical and genetic signaling and further regulate specific cellular functions. This process is known as mechanochemical

transduction or mechanotransduction (Fig. 1).<sup>14-16,19-21,25-27</sup> Mechanotransduction involves a great number of mechanosensors or mechanosensitive biomolecules, such as integrin, cadherin, Piezo 1/2, G-protein-coupled receptor (GPCR), YAP/ transcriptional co-activator with PDZ-binding motif (TAZ), Wnt, mitogen-activated protein kinase (MAPK)/extracellular-signalregulated kinase (ERK), and phosphoinositide 3-kinase (PI3K)/ protein kinase B (AKT), which experience conformational and functional changes under mechanical stimuli.19,45-49 For example, cytoskeleton, such as filamentous (F)-actin, microtubules (MTs), and intermediate filaments (IFs), and their associated motor proteins, such as myosin II, act as molecular connectors to transmit forces directly from the extracellular environment, through the membrane mechanosensors, into the nucleus.<sup>31</sup> Unlike diffusion-based chemical signal propagation, this force/deformation-based mechanical signal transmission can modulate the nucleus and affect gene activation within milliseconds.<sup>50-53</sup> Dysregulated mechanotransduction often results in diseases.54-58

Emerging studies have demonstrated the functional roles of mechanical cues in tumor progression at different stages (Fig. 1).<sup>7,9,11–17,59–62</sup> Several aspects of the biophysical microenvironment in tumors are dramatically altered compared to their healthy counterparts, such as tissue stiffness,<sup>63</sup> solid stress,<sup>64</sup> interstitial fluid pressure,<sup>65</sup> cell stiffness,<sup>66,67</sup> cell contractility,<sup>68,69</sup> and cell adhesion.<sup>70–72</sup> These altered biophysical signals are transmitted into tumor cells *via* mechanotransduction and mediate cancer pathobiology. Understanding the molecular mechanisms of mechanotransduction in tumor development has inspired and enabled researchers to design and develop novel cancer therapies.<sup>40,73</sup> In the following sections, we focus on three critical means of biochemical signaling in cancer: Ca<sup>2+</sup>, YAP, and miRNAs, and address their functions and crosstalk.

Ca<sup>2+</sup> is a universal and indispensable signaling ion used by all eukaryotes.<sup>74–77</sup> In tumor cells, Ca<sup>2+</sup> regulates cellular activities and impact tumor progression including proliferation, metabolism, migration, epithelial–mesenchymal transition (EMT), and apoptosis.<sup>78–82</sup> Emerging studies have demonstrated that Ca<sup>2+</sup> signaling in cancer cells is affected by various mechanical stimuli including cyclic stretch,<sup>83</sup> local membrane traction,<sup>84</sup> fluid shear,<sup>85,86</sup> and compression.<sup>87,88</sup> Because the force-regulated Ca<sup>2+</sup> signals have critical roles in cancer progression, the understanding of their underlying molecular mechanisms can provide insights into the development of novel cancer therapies. However, how mechanical stimuli convert into and regulate Ca<sup>2+</sup> signals *via* mechanotransduction pathways has not been systematically studied.

YAP is a protein that can bind to transcription factors in the nucleus to regulate cellular functions.<sup>89,90</sup> In cancer, expression and nucleus accumulation of YAP regulate tumor cell initiation, proliferation, migration, stemness, and chemoresistance.<sup>91–94</sup> YAP's nucleus/cytoplasm distribution is sensitive to mechanical stimuli that cells experience, such as substrate stiffness, cyto-skeleton tension, nuclear deformation, and extracellular mechanical tension/compression.<sup>95</sup> However, how YAP responds to

mechanical stimuli at molecular levels is still being actively investigated.<sup>96–99</sup> Importantly, YAP activation is necessary in tumor initiation of squamous cell carcinoma and uveal melanoma<sup>100,101</sup> and promotes the dissemination of circulating tumor cells.<sup>102</sup> Aberrant YAP expression in different cancer types and its regulatory roles on the cancer progression necessitate the mechanistic dissections of how mechanical cues regulate YAP activity, which in turn contribute to the development of YAP-targeted anti-cancer mechano-medicine.

miRNAs are small non-coding RNAs that regulate geneexpression post-transcriptionally.<sup>103,104</sup> Increasing evidence has demonstrated that miRNAs participate in the modulation of cancer-related pathways, from which diverse miRNAs have been used as diagnostic biomarkers and therapeutic targets/ agents in anti-cancer treatments.<sup>105,106</sup> How miRNAs can be potentially exploited for targeting calcium signaling, YAP activities, and mechanotransduction in cancer therapy is now being actively studied.

## 3. Calcium (Ca<sup>2+</sup>) signals in cancer

# 3.1 Significance of Ca<sup>2+</sup> signals in tumor initiation, development, and progression

Calcium signals have pivotal roles in regulating cancer progression at different stages: tumor initiation, growth, angiogenesis, metastasis, and colonization (Fig. 2 and Table 1).<sup>79,107,108</sup>

During cancer initiation, altered calcium signals, due to aberrant expression and activities of Ca2+ channels/transporters, lead to abnormal cellular functions, such as defects in autophagy<sup>109</sup> and resistance to apoptosis.<sup>110</sup> Because autophagy functions as a tumor suppressor,<sup>111–113</sup> both uncontrolled increases and decreases of cytoplasmic Ca2+ concentrations ([Ca<sup>2+</sup>]<sub>cvt</sub>) can cause defects in autophagy to break normal cellular homeostasis and favor cancerous phenotypes.<sup>109</sup> In addition, p53-deficient cells fail to induce mitochondrial Ca<sup>2+</sup> overload via endoplasmic reticulum (ER)-mitochondrial Ca<sup>2+</sup> signaling and become apoptosis-resistant.<sup>110</sup> This selection advantage of p53-deficiency favors survival of damaged cells, potentially resulting in cancer initiation. Moreover, altered  $[Ca^{2+}]_{cvt}$  that is mediated by a great number of  $Ca^{2+}$ channels and Ca<sup>2+</sup>-binding proteins contributes to increased cancer cell stemness and tumorigenesis.<sup>114–119</sup>

During tumor growth, increased expression and activities of  $Ca^{2+}$  channels in cancer cells raise  $[Ca^{2+}]_{cyt}$  to levels above those of healthy cells, and lead to uncontrolled, elevated cell proliferation by regulating downstream effectors in multiple key stages of the cell cycle.<sup>79,84,120–122</sup> In addition, intracellular calcium signaling regulates tumor growth by modulating cancer cell death, partly through autophagy or mitochondrial  $Ca^{2+}$  overload.<sup>123–127</sup> During angiogenesis in solid tumors, calcium signals regulate the proliferation and migration of vascular endothelial cells. Vascular endothelial growth factor (VEGF)- or basic fibroblast growth factor (BFGF)-induced increases of  $[Ca^{2+}]_{cyt}$  activates proliferation of vascular endothelial cells in solid tumors.<sup>79,128</sup> Both elevated and reduced  $Ca^{2+}$ 



Fig. 2 Significance of calcium signals during cancer initiation, development, and progression.

influxes can enhance the migration of tumor-derived endothelial cells (TECs) compared to normal endothelial cells (NECs), which results in abnormal tumor vasculature.<sup>86,129,130</sup>

EMT is the cellular process of acquiring mesenchymal features from epithelial cells, causing tumor cells to become invasive and metastatic.<sup>62,131,132</sup> At the early stages of EMT and metastasis, calcium signals are required.133-135 During metastasis, diverse calcium signaling pathways are regulated by transient receptor potential (TRP) channels, inositol trisphosphate receptors (IP<sub>3</sub>Rs)/rvanodine receptors (RvRs), voltagegated calcium channels (VGCCs), and store-operated Ca<sup>2+</sup> entry (SOCE).<sup>81</sup> These pathways (1) modulate local  $[Ca^{2+}]_{cvt}$  at distinct intracellular regions of cancer cells and (2) regulate Ca2+dependent effectors for the formation or turnover of focal adhesions, thus facilitating migration.87,88,122,136-140 Calcium signaling including SOCE is proposed to remodel distant sites and facilitate the colonization of secondary tumors in new organs by assisting metastasized tumor cells to exploit growth factors embedded in the extracellular matrix (ECM).<sup>141</sup> SOCEenhanced secretion of VEGF and prostaglandins E2 from primary tumors may mobilize angiogenesis at distant organs to form pre-metastatic niches. In addition, Ca<sup>2+</sup> itself serves as a chemoattractant of tumor cells for bone colonization.<sup>142-145</sup>

Readers are referred to Table 1 for the specific influence of altered  $Ca^{2+}$  signals on cancer development. Next, we will discuss how mechanical stimuli can influence and regulate  $[Ca^{2+}]_{cyt}$  via the interplay between mechanotransduction and  $Ca^{2+}$  signaling pathways.

# 3.2 Intra-/inter-cellular calcium responses induced by mechanical stimuli

**3.2.1 Microenvironmental stiffness.** Increasing evidence demonstrates that the mechanical microenvironment mediates Ca<sup>2+</sup> signaling in non-cancer cells (Fig. 3A), such as human mesenchymal stem cells (HMSCs),<sup>146,147</sup> fibroblasts,<sup>148</sup> neutrophils,<sup>149</sup> myofibroblasts,<sup>150</sup> macrophages,<sup>151</sup> and human neuronal

progenitor cells.<sup>152</sup> HMSCs cultured on rigid dishes (Young's modulus:  $E \sim 3$  GPa) have been observed to generate spontaneous [Ca<sup>2+</sup>]<sub>cvt</sub> oscillations.<sup>147</sup> When the substrate stiffness is lowered to 1 kPa, the signal amplitudes and frequencies of the Ca<sup>2+</sup> oscillations are reduced in a Ras homolog family member A (RhoA)/Rho-associated protein kinase (ROCK)-dependent manner. During the cell-matrix adhesion process, HMSCs on 40 kPa substrates show more [Ca<sup>2+</sup>]<sub>cvt</sub> increase at detergent-resistant membrane (DRM) microdomains than those on 0.6 kPa substrates.146 Focal adhesion kinase (FAK) and mechanosensitive transient receptor potential melastatin 7 (TRPM7) mediate this substrate-rigidity-dependent Ca2+ signal. In 2D-cultured mouse fibroblasts, a larger percentage of cells cultured on soft 690 Pa substrates show Ca2+ oscillations in response to adenosine-5'triphosphate (ATP) than those on intermediate stiff 36 kPa substrates, through F-actin-mediated mechanotransduction.148 Similar mechanobiological effects on cellular Ca2+ responses are observed in 3D-cultured mouse fibroblasts. Neutrophils show mechanical-microenvironment-dependent calcium spikes when adhered on the human umbilical vascular endothelial cell (HUVEC) monolayer that is pre-formed on stiffness-varied substrates.<sup>149</sup> On stiffer glass substrates ( $E \sim 70$  GPa), HUVECs demonstrate a higher average cell stiffness of 13.10 kPa, leading to increased [Ca<sup>2+</sup>]<sub>cvt</sub> and spike frequency in the neutrophils. The mechanism of this rigidity-enhanced calcium response is associated with selectin-induced  $\beta_2$ -integrin activation and actin polymerization in cells. The stiffer substrates enhance the polymerization of F-actin that pushes the plasma membrane to increase membrane tension and open mechanosensitive Ca2+ channels. In myofibroblasts<sup>150</sup> and macrophages,<sup>151</sup> stiffer matrices augment agonist-induced Ca<sup>2+</sup> influx via mechanosensitive transient receptor potential vanilloid 4 (TRPV4) channels. Human neuronal progenitor cells cultured on stiffer substrates are more responsive to the activation of GPR68, which is a mechanosensitive GPCR and triggers ER  $Ca^{2+}$  release via the Gq-phospholipase C (PLC)-IP<sub>3</sub>R pathway to regulate the Ca<sup>2+</sup>

Table 1 Significance of calcium signals in tumor progression

Impact on cancer	Specific Ca <sup>2+</sup> signal	Upstream	Downstream	Exact Influence	Cell type	In vitro/ vivo?	t Matte
Initiation	Increased [Ca <sup>2+</sup> ] <sub>ort</sub>	Ca <sup>2+</sup> influx <i>via</i> TRPV2	Dysregulation of the Wnt/ β-catenin signaling pathway	Induced Ca <sup>2+</sup> influx inhibits stemness of cancer cells	Human bone osteosarcoma, breast, and colorectal adenocarcinoma	In vitro & in vivo	117 5
	Elevated Ca <sup>2+</sup> current	Overexpressed L- and T-type Ca <sup>2+</sup> channels	AKT and ERK signaling pathways	Downregulation of $Ca^{2+}$ channels reduces the expression of stemness markers	ovarian cancer stem cells	In vitro & in vivo	119
Growth	IP <sub>3</sub> R3-BK <sub>Ca</sub> coupled Ca <sup>2+</sup> signaling	ATP-induced ER Ca <sup>2+</sup> release <i>via</i> IP <sub>3</sub> R3	Activation of BK <sub>Ca</sub> channel on plasma membrane	IP <sub>3</sub> R3/BK <sub>Ca</sub> silencing impairs ATP-increased cell proliferation (arrested in G0/G1 nhase)	Human breast cancer cells	In vitro	121
	Spontaneous [Ca <sup>2+1</sup> ] <sub>yt</sub> oscillations; 80% cancer cells showing oscillation vs. 30% non-cancer cells	Increased expression and activity of Orai1	Downregulation of cdc2, Cyclin B1, and p27	proliferation, migration, invasion, and tumor growth	Human esophageal squamous cell carcinoma	In vitro & in vivo	122
	Elevated [Ca <sup>2+</sup> ] <sub>cyt</sub>	Ca <sup>2+</sup> influx <i>via</i> Piezo1	Akt/mTOR pathway; activa- tion of CDK4 and cyclin D1	Downregulation of Piezo1 sup- presses cell proliferation and tumor ortowth	Human prostate cancer cells	In vitro & in vivo	84
	Decreased ATP-induced [Ca <sup>2+</sup> ] <sub>cyt</sub> elevation	IP <sub>3</sub> R inhibition/silencing	Induced autophagy	IP <sub>3</sub> R inhibition induces cell death and suppresses tumor growth	Human breast cancer	In vitro & in vivo	123
	Reduced SOC current	Decreased Orai1 expression	Inhibited apoptosis- inducing pathways	Downregulation of Orai1 protects	Human prostate cancer	In vitro	124
	Enhanced ER-mitochondrial Ca <sup>2+</sup> signaling	p53 binding to SERCA $\Rightarrow$ ER Ca <sup>2+</sup> overload $\Rightarrow$ ER Ca <sup>2+</sup> release	Mitochondrial Ca <sup>2+</sup> overload $\Rightarrow$ alteration of mitochondrial mornholoov	terms trout apoptessor p53 induces apoptosis	Human colorectal, cervical, lung cancer cells	In vitro	125
	<i>K-Ras<sup>613D</sup></i> deletion-enhanced agonist-induced ER Ca <sup>2+</sup> release	Remodeled IP <sub>3</sub> R expression and increased SERCA2b expression ⇒ increased ER Ca <sup>2+</sup> content	Increased mitochondrial Ca <sup>2+</sup> uptake	Deletion of <i>K-Ras<sup>G13D</sup></i> sensitizes cells to apoptosis	Human colorectal cancer cells	In vitro	126
	Increased ER Ca <sup>2+</sup> release	BRCA1 binding to $IP_3R \Rightarrow$ regulation of $IP_3R$ function	Not through mitochondrial Ca <sup>2+</sup> overload	Tumor suppressor BRCA1 is recruited for apoptosis	Human cervical, ovar- ian cancer cells	In vitro	127
Angiogenesis	Elevated [Ca <sup>2+</sup> ] <sub>cyt</sub>	$\mathrm{Triclosan} \Rightarrow \mathrm{TRPA1}$	VEGF secretion	Triclosan stimulates epithelial cell proliferation	Human prostate cancer stromal cells	In vitro	128
	Induced [Ca <sup>2+]</sup> <sub>byt</sub> transients	$Ca^{2+}$ influx <i>via</i> TRPV4	Migration-related signaling	TRPV4 activation enhances migra- tion of endothelial cells	Tumor-derived endo- thelial cells from human breast	In vitro	129
	Reduced [Ca <sup>2+</sup> ] <sub>cyt</sub> elevation	Decreased TRPV4 expression	High Rho activity	TRPV4 activation restores mechan-	carcinomas Tumor-derived endo-	In vitro	130
				osensitivity and inhibits migration of endothelial cells, and normalizes	thelial cells from an adenocarcinoma	& in vivo	
EMT	EGF/scratch-induced transient [Ca <sup>21</sup> ] <sub>9vt</sub> increase (2-fold higher)/Ca <sup>2+</sup> wave	Ca <sup>2+</sup> influx <i>via</i> TRPM7 (mechanosensitive) and other Ca <sup>2+</sup> channels	STAT3 phosphorylation and vimentin expression; induc- tion of Twist, N-cadherin, CD44/CD24	hypoxia-induced EMT (biomarkers)	Human breast cancer cells	In vitro	135
	Intracellular Ca <sup>2+</sup> elevation	Ca <sup>2+</sup> influx <i>via</i> acid-sensing ion channels	Upregulation of RhoA activity	Inhibition of Ca <sup>2+</sup> influx or intra- cellular Ca <sup>2+</sup> chelation suppresses induced FMT	Pancreatic cancer cells	In vitro & in vivo	133
	Increased SOCE	Activation of STIM	EMT-related signaling	STIM-mediated SOCE facilitates	Human breast cancer	In vitro	134 Sev
Metastasis	Reduced [Ca <sup>2+</sup> ] <sub>cyt</sub> elevation	Low TRPM7 activity	Inactivated RhoA/myosin-II and IQGAP1-Cdc42		Human fibrosarcoma cells		view

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Table 1 (con	tinued)						
Impact on cancer	Specific Ca <sup>2+</sup> signal	Upstream	Downstream	Exact Influence	Cell type	In vitro/ vivo?	Ref.
	[Ca <sup>2+]</sup> <sub>cyt</sub> elevation	Induced Ca <sup>2+</sup> influx <i>via</i> TRPV4	Accelerated actin dynamics and downregulated cytoskeleton-associated pro-	Reduced TRPM7-mediated Ca <sup>2+</sup> influx inhibits shear flow sensing and facilitates intravasation Overexpression of TRPV4 increases cell invasiveness	Human breast cancer cells	In vitro & in vivo In vitro & in vivo	137
	$[Ca^{2+}]_{cyt}$ elevation	Ca <sup>2+</sup> influx <i>via</i> Piezo1	teins at the cell cortex Protrusions of apical actin and expression of MMP-9	Piezo1-mediated Ca <sup>2+</sup> influx enhances cell invasion and matrix	Human breast cancer cells	In vitro	88
	Sustained $[Ca^{2+}]_{cyt}$ elevation	Induced ER Ca <sup>2+</sup> release <i>via</i> 17-R3	Migration-related signaling	degradation Overexpression of IP <sub>3</sub> R3 enhances ATD-induced cell micration	Human breast cancer	In vitro	138
	Induced Ca <sup>2+</sup> responses	$GPCR/RTK \Rightarrow PLC \Rightarrow IP_3R$	Migration-related signaling	Caffeine inhibition of IP <sub>3</sub> R blocks glioblastoma invasion and	Human glioblastoma	In vitro & ·	139
	[Ca <sup>2+</sup> ] <sub>cyt</sub> elevation	ER Ca <sup>2+</sup> release <i>via</i> IP <sub>3</sub> R	Promoted cortical actomyo- sin contractility	Increased survival of mouse model Nuclear envelope tension induced ER Ca <sup>2+</sup> release facilitates cell	Human cervical carcinoma cells	ın vivo In vitro	87
	Spontaneous [Ca <sup>2+]</sup> <sub>3v1</sub> oscilla- tions; 80% cancer cells showing oscillation vs. 30% non-cancer cells	Increased expression and activity of Orai1	Increased expression of vimentin and Rac1; down- regulation of E-cadherin	transmitteration unough on mattra KD of Orai1 inhibits cell proliferation, migration, invasion, and tumor growth	Human esophageal squamous cell carcinoma	In vitro & in vivo	122
	Spontaneous periodic intra- cellular propagations of peri- membrane $Ca^{2x}$ waves (freq = 3 times min <sup>-1</sup> ;	Low voltage-activated T-type Ca <sup>2+</sup> channels and non- voltage-gated cation channels (on hasma membrane)	Migration-related signaling	Block of Ca <sup>2+</sup> signals reduces cell motility and invasion	Human fibrosarcoma cells	In vitro	140
Colonization	[Ca <sup>2+</sup> ] <sub>cyt</sub> elevation	Ca <sup>2+</sup> flow <i>via</i> gap junctions	Enriched NEAT and MEF2 activities	$Ca^{2+}$ flow from osteogenic cells to cancer cells facilitates bone colonization	Human breast cancer cells	In vitro & in vivo	145



**Fig. 3** Biophysical cues regulate calcium signaling. (A) Stiff substrates enhance intracellular calcium signaling *via* RhoA/ROCK pathway,<sup>147</sup> integrin/FAK/ actin mechanotransduction,<sup>146,149</sup> mechanosensitive ion channels,<sup>146,149–151</sup> and mechanosensitive-GPR68-triggered Gq-PLC-IP<sub>3</sub>R pathway.<sup>152</sup> Soft substrates enhance calcium oscillations in mouse fibroblasts in an F-actin-dependent manner.<sup>148</sup> (B) Mechanical stretch from substrates enhances intracellular calcium signaling *via* actomyosin contraction,<sup>156,157</sup> mechanosensitive ion channels,<sup>83,156,157</sup> mechanosensitive GPR68,<sup>152</sup> PLC-IP<sub>3</sub>R signaling pathway,<sup>156,157</sup> and ER-NE tension.<sup>154</sup> (C) Local membrane tension enhances intracellular calcium signaling *via* mechanosensitive ion channels<sup>84,158</sup> and cytoskeletal-mechanotransduction-regulated ER calcium release.<sup>158</sup> (D) Fluid shear stress enhances intracellular calcium signaling *via* mechanosensitive ion channels<sup>85,136</sup> and mechanosensitive-GPCR-triggered Gq-PLC-IP<sub>3</sub>R pathway.<sup>86,159</sup> (E) Mechanical compression enhances intracellular calcium signaling *via* mechanosensitive ion channels,<sup>88</sup> ER-NE tension,<sup>87</sup> and SOCE.<sup>162</sup> (F) Mechanical stimuli regulate the expression and function of connexin-based gap junctions<sup>178–180</sup> and hemichannel-/exocytosis-regulated ATP release,<sup>181,183–189</sup> which are major pathways for intercellular propagation of calcium waves. The scale bar in (A) represents 5 µm length and applies to all other subfigures.

response.<sup>152,153</sup> These data highlight that microenvironment mechanics critically regulates  $[Ca^{2+}]_{cyt}$  through diverse  $Ca^{2+}$  signaling pathways.

cancer<sup>83</sup> and non-cancer cells<sup>152,154–157</sup> (Fig. 3B). In human breast cancer cells, cyclic stretch causes Ca<sup>2+</sup> influx *via* Piezo1 channels in a strain amplitude- and frequency-dependent manner.<sup>83</sup>

**3.2.2 Mechanical stretch from environments.** Active mechanical stretch triggers intracellular  $Ca^{2+}$  signals in both

In HMSCs, prolonged stretch triggers intracellular Ca<sup>2+</sup> oscillations.<sup>156</sup> This Ca<sup>2+</sup> response is dependent on calcium

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influx via mechanosensitive Ca2+ channels on the plasma membrane, as well as the cytoskeleton, actomyosin contractility, and PLC activity. In HUVECs, vibrational stretch triggered global (80%) and local (20%) intracellular Ca<sup>2+</sup> responses.<sup>157</sup> The global [Ca<sup>2+</sup>]<sub>cyt</sub> increase is regulated by mechanosensitive Ca<sup>2+</sup> channels on the plasma membrane, PLC-IP<sub>3</sub>R signaling pathway, and the resultant ER Ca2+ release, as well as F-actin assembly and actomyosin contractility. In the monolaver of human epidermal stem/progenitor cells (EPCs), cyclic stretch induces intracellular Ca<sup>2+</sup> flashes.<sup>154</sup> The underlying mechanism involves stretch-triggered nuclear deformation and ER-nuclear envelope (NE) tension, which causes Ca<sup>2+</sup> release from the ER. Mechanically stretched human neuronal progenitor cells are more responsive to the activation of mechanosensitive GPR68 and show higher and faster elevation of [Ca<sup>2+</sup>]<sub>cvt</sub> compared to unstretched cells.<sup>152</sup> Meniscus fibrochondrocytes (MFCs) (1) within the native tissues, (2) on aligned nanofibrous scaffolds, and (3) on silicone membranes, all show a baseline level of intracellular Ca<sup>2+</sup> oscillations.<sup>155</sup> Larger tensile deformation of all three types of substrates increases the population of cells that show intracellular Ca<sup>2+</sup> oscillations, with the characteristics of a linear increase below 3% strain and a gradual plateau over 6%. The working mechanisms remain to be identified.

**3.2.3 Local mechanical tension on cell membrane.** Local membrane tension triggers intracellular Ca<sup>2+</sup> signals in human prostate cancer cells<sup>84</sup> and HMSCs<sup>158</sup> (Fig. 3C). Mechanosensitive Piezo1 channels are highly expressed in human prostate cancer PC3 and DU145 cell lines and in human prostate malignant tumor tissues.<sup>84</sup> In DU145 cells, mechanical stimulation by heat-polished glass probes induces Ca<sup>2+</sup> influx *via* Piezo1. Gene knockdown and pharmacological data reveal that these Piezo1-regulated intracellular Ca<sup>2+</sup> signals are influential to cancer cell proliferation and migration *in vitro* and to prostate tumor growth *in vivo*.<sup>84</sup>

In HMSCs, laser-tweezer-induced tension at the plasma membrane triggers intracellular  $Ca^{2+}$  oscillations.<sup>158</sup> The underlying mechanisms involve (1)  $Ca^{2+}$  influx *via* mechanosensitive TRPM7 channels, which is dependent on passive cytoskeletal support of F-actin and microtubules, and (2) ER  $Ca^{2+}$  release, which is dependent on cytoskeletal structure, actomyosin contractility, and TRPM7 activity. These data reveal that cytoskeleton indeed transmits mechanical signals from cell membrane into intracellular organelles and regulate  $Ca^{2+}$ signaling pathways.<sup>158</sup>

**3.2.4 Fluid shear stress.** Fluid shear stress induces intracellular Ca<sup>2+</sup> signals in human cancer cells,<sup>85,86</sup> HUVECs,<sup>159</sup> and normal human fibroblasts<sup>136</sup> (Fig. 3D). Fluid shear stress of 2.0 dyn cm<sup>-2</sup> sensitizes human prostate cancer cells to tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis.<sup>85</sup> Piezo1 channels introduce calcium influx to activate apoptotic pathways and regulate the forceinduced TRAIL sensitization. In human breast cancer cells, fluid shear stress of 2 Pa elevated  $[Ca^{2+}]_{cyt}$  in a Ca<sup>2+</sup>-store-dependent manner through the activation of mechanosensitive GPR68.<sup>86</sup> In HUVECs, shear stress induces calcium transients by activating mechanosensitive GPCR, H<sub>1</sub>R.<sup>159</sup> Compared to normal fibroblasts, human fibrosarcoma cells show reduced TRPM7 current in response to shear flow that is present in the vasculature, which facilitates cancer cell intravasation.<sup>136</sup> Overexpressing the mechanosensitive TRPM7 sensitizes cancer cells to shear flow and attenuates invasion out of the primary tumor, intravasation, and metastatic lesion formation. In contrast, human normal fibroblasts show shear-stress-triggered Ca<sup>2+</sup> influx *via* TRPM7 and the downstream activation of RhoA/myosin-II and calmodulin/ IQGAP1/Cdc42 pathways, which reverses the direction of cell migration to avoid shear flow.

3.2.5 Mechanical compression. In a solid tumor, intratumoral residual or solid stress builds up due to physical resistance from the surrounding healthy tissue against the outgrowth of tumor cells,<sup>64</sup> causing mechanical compression with magnitudes of 0.002-20 kPa.<sup>64,160,161</sup> Compression induces intracellular Ca<sup>2+</sup> signals in human cancer cells<sup>87,88</sup> and zebrafish embryonic progenitor cells<sup>162</sup> (Fig. 3E). In human breast cancer cells, vertical compressive stress at magnitudes of 400 Pa and 600 Pa induces Ca<sup>2+</sup> influx via mechanosensitive Piezo1 channels, which enhances cancer cell invasion via invadopodia formation and matrix degradation.<sup>88</sup> In human cervical carcinoma cells, compressive stress causes nuclear deformation and ER-NE tension to trigger ER Ca<sup>2+</sup> release via stretch-sensitive Ca<sup>2+</sup> channels IP<sub>3</sub>Rs.<sup>87</sup> The resultant elevation of [Ca<sup>2+</sup>]<sub>cvt</sub> facilitates cancer cell transmigration through 3D collagen lattices and synthetic pores, which is attenuated by IP<sub>3</sub>R antagonist 2-aminoethoxydiphenyl borate (2-APB) and Xestospongin C. In zebrafish embryonic progenitor cells, compression-induced nuclear deformation elevated intracellular Ca<sup>2+</sup> concentrations, with a specific increase of Ca<sup>2+</sup> in the nucleus.<sup>162</sup> This specific Ca<sup>2+</sup> signaling involves SOCE, which consists of ER Ca<sup>2+</sup> sensor stromal interaction molecule (STIM) and Orai Ca<sup>2+</sup> channels on plasma membrane.

3.2.6 Mechano-regulatory cell-cell communication by Ca<sup>2+</sup> signaling. In cancer cells, the molecular mechanisms of intercellular Ca<sup>2+</sup> wave (CW) propagation are mainly (1) gapjunction-based, involving internal transportation of Ca<sup>2+</sup>/ inositol trisphosphate  $(IP_3)^{163-165}$  and (2) paracrine-based, involving extracellular diffusion of ATP<sup>166-168</sup> (Fig. 3F). Elevation of cytoplasmic concentration of Ca<sup>2+</sup> or IP<sub>3</sub> results in diffusion of the ions/molecules into neighboring cells via gap junctions.<sup>169</sup> The diffused Ca<sup>2+</sup> ions further activate IP<sub>3</sub>Rs and RyRs on the ER membrane to trigger calcium-induced calcium release (CICR) signaling, while IP3 opens only the IP<sub>3</sub>R channel.<sup>153</sup> In MCF-7 human breast cancer cells, photoexcitation-induced cytosolic Ca<sup>2+</sup> release triggers propagation of CWs in 2D-cultured contacted and non-contacted cells, as well as in 3D-cultured contacted cells.<sup>163</sup> The CW propagation in 2D-contacted cells requires the functional gap junctions. In HeLa cells, the transfer of IP<sub>3</sub> through connexin 43 (Cx43)based gap junctions is required for intercellular communication of Ca<sup>2+</sup> signals *via* tunneling membrane nanotubes, in the absence of paracrine transmission.<sup>164</sup> The photolysis of intracellular caged-IP3 triggers the propagation of CWs in C6 glioma cells expressing Cx43 or Cx32, but not in gap-junctiondeficient cells.165

Intracellular ATP can be released to the extracellular milieu *via* (1) channels including hemichannels, maxi-anion channels and P2X7 receptors,<sup>169–172</sup> (2) ATP-binding cassette (ABC) transporters,<sup>170,173,174</sup> (3) exocytosis,<sup>169,170</sup> and (4) lysis.<sup>170,174</sup> ATP activates membrane (1) P2Y receptors and the downstream Gq-PLC-IP<sub>3</sub>R pathway to induce Ca<sup>2+</sup> release from the ER,<sup>175,176</sup> and (2) P2X receptors to trigger the influx of extracellular cations including Ca<sup>2+</sup>.<sup>176,177</sup> In lung and prostate cancer cells, the mechanical-injury-triggered propagation of CWs in cultured epithelial layers is ATP-dependent.<sup>166</sup> In non-contacted MCF-7 cells, the mechanically stimulated intercellular propagation of CWs involves extracellular ATP release.<sup>167</sup> In HeLa cells, mechanically induced intercellular CWs involve both connexinbased gap junctions and extracellular ATP in a convoluted manner.<sup>168</sup>

The expression and function of connexin-hemichannelformed gap junctions are responsive to mechanical stimuli, which further regulate intercellular communication.<sup>178-180</sup> Certain connexins including Cx43 are sensitive to several types of mechanical stimuli, such as cyclic stretch, static tension, and shear stress. Moreover, ATP release can be stimulated by different types of mechanical stimuli including osmotic pressure, fluid shear stress, substrate stretch, compression, and injury.<sup>181,182</sup> ATP-releasing connexin<sup>183,184</sup> and pannexin<sup>185</sup> hemichannels are mechanosensitive and further induces intercellular Ca<sup>2+</sup> signaling.<sup>186,187</sup> In addition, mechanical stretch,<sup>188</sup> fluid shear stress,<sup>183,189</sup> and injury<sup>181</sup> induce exocytosis of ATP-containing vesicles in a Ca<sup>2+</sup>-dependent manner.

Overall, intra- and inter-cellular  $Ca^{2+}$  signaling can be triggered and enhanced by various types of mechanical stimuli, which have functional roles during tumor progression. We next review the current understanding of the molecular mechanisms of the mechano-regulated  $Ca^{2+}$  signaling pathways and the role of cytoskeletal proteins during this process.

# 3.3 Molecular mechanisms of the crosstalk between calcium signaling pathways and cytoskeletal proteins

3.3.1 [Ca<sup>2+</sup>]<sub>cyt</sub> and cytoskeletal proteins. In cancer cells, diverse Ca<sup>2+</sup> signaling pathways regulate cell migration and metastasis by directly and/or indirectly targeting cytoskeletal proteins and adhesion molecules.<sup>81,190</sup> In HeLa cells, chelation of intracellular Ca<sup>2+</sup> attenuates F-actin, increases filopodia formation, and reduces the size and number of focal adhesions.<sup>191</sup> In prostate cancer cells that have high metastatic capacity, enhanced ATP-induced Ca<sup>2+</sup> transients correlate with higher occurrences of actin proteins anchoring at focal adhesion sites, which is revealed by quantitative co-localization of the spatial distributions between actin and vinculin.<sup>192</sup> In prostate cancer cells, inhibition of calcium/calmodulindependent protein kinase II (CaMKII), which is a transducer of Wnt/Ca<sup>2+</sup> signaling, remodels actin cytoskeleton and increases the frequency and length of filopodia protrusions, leading to reduced cell motility for wound closure.<sup>193</sup>

In non-cancer cells, the actin cytoskeleton and its associated proteins regulate Ca<sup>2+</sup> signaling.<sup>194,195</sup> Actin cytoskeleton<sup>196</sup>

and cortical actin<sup>197,198</sup> modulate Ca<sup>2+</sup> flashes/waves during egg activation and fertilization.

3.3.2 Mechanosensitive ion channels and cytoskeletal proteins. In Section 3.2, we summarized how (1) mechanosensitive ion channels, such as Piezo1,<sup>83-85,88</sup> TRPM7,<sup>136,146,158</sup> and TRPV4,<sup>130,150,151</sup> and (2) GPCRs, such as GPR68<sup>86,152</sup> and H<sub>1</sub>R<sup>159</sup> can sense mechanical stimuli and trigger Ca<sup>2+</sup> signaling. Reciprocally, Ca<sup>2+</sup> signaling induced by mechanosensitive ion channels affects cytoskeleton remodeling.88,136,137,199,200 In MDA-MB-231 human breast cancer cells, Piezo1-regulated Ca<sup>2+</sup> influx promotes the formation of cortical stress fibers and protrusions of apical actin.<sup>88</sup> In brain metastases of human breast cancer cells (MDA-MB-231-BrM2), mechanosensitive channel Piezo2 induces Ca<sup>2+</sup> influx to activate RhoA, which further regulates the formation of actin cytoskeleton and the orientation of focal adhesions.<sup>200</sup> In MB468 human breast cancer cells, transfected TRPV4 increases the average globular (G)- to F-actin ratio by 22% and reduces the phospho-Cofilin expression level by 1.45-fold.<sup>137</sup> In human fibroblasts, TRPM7regulated Ca<sup>2+</sup> influx activates myosin-II contractility via the RhoA/myosin-II pathway to modulate migration direction.<sup>136</sup>

In MDA-MB-231 human breast cancer cells, cyclic mechanical stretch induces a higher level of Piezo1-mediated  $Ca^{2+}$ influx than that in MCF10A normal human breast cells.<sup>83</sup> In another study in MDA-MB-231 cells, Piezo1 is expressed in the cytoplasm including the plasma membrane, but in MCF10A cells, it is mainly expressed in the nuclear region, especially the nuclear envelope.<sup>201,202</sup> This trait is likely to contribute to the different stretch-triggered  $Ca^{2+}$  responses between MDA-MB-231 cells and MCF10A cells. In the same study, expression of tropomyosin 2.1 (TPM2.1) is found in MCF10A cells but not in MDA-MB-231 cells, which is responsible for the different levels of stretch-induced  $Ca^{2+}$  influx in the two cell types.<sup>83</sup> The data indicate that TPM2.1 regulates the expression location of Piezo1 in human breast cancer and normal cells.

In TECs from human breast carcinomas (BTECs), arachidonic acid (AA) treatment triggers actin remodeling and increases TRPV4 expression on the plasma membrane.<sup>129</sup> Following preincubation of BTECs with AA, TRPV4 predominantly traffic from the cytoplasm to the cell membrane, demonstrating colocalization with the cortical actin in the cell periphery. In contrast, in the control untreated BTECs group, TRPV4 and actin mostly diffuse in the cytoplasm. The data indicate that the actin cytoskeleton interacts with TRPV4 channels and regulates the expression location of TRPV4 in BTECs.

**3.3.3 IP<sub>3</sub>R and cytoskeletal proteins.** IP<sub>3</sub>Rs are intracellular ligand-gated Ca<sup>2+</sup>-release channels, mainly expressed on the ER membrane.<sup>153,203,204</sup> IP<sub>3</sub>Rs have important roles in cancer by regulating cell autophagy, apoptosis, proliferation, migration, and invasion.<sup>203,205,206</sup>

In cancer cells, IP<sub>3</sub>Rs regulate cytoskeleton remodeling. In human breast cancer cells, IP<sub>3</sub>R3 mediates intracellular Ca<sup>2+</sup> signaling and remodels profilin cytoskeleton *via* the ARHGAP18/RhoA/mDia1/FAK pathway.<sup>207</sup> IP<sub>3</sub>R3 silencing causes oscillatory characteristics of [Ca<sup>2+</sup>]<sub>cyt</sub> signals after ATP administration or wound formation and alters the localization

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of F-actin and expression level of profilin. The remodeling of cytoskeletal proteins decreases cancer cell adhesion to collagen I-coated wells and induces rounded cell shape. However, how cytoskeletal proteins regulate IP<sub>3</sub>Rs and the downstream Ca<sup>2+</sup> signaling in cancer is less known. To the best of our knowledge, only one study specifically reported that KRAS-induced actininteracting protein (KRAP) is involved in the modulation of IP<sub>3</sub>R-regulated ER Ca<sup>2+</sup> release in MCF7 breast cancer cells.<sup>208</sup> Knockdown of KRAP attenuates the amplitude of ATP-induced Ca<sup>2+</sup> release by 12–32% (peak response) in an ATP-concentration-dependent manner. KRAP is associated with IP<sub>3</sub>Rs in HCT116 colon cancer and HeLa cervical cancer cells, as well as in mouse liver and pancreas tissues.<sup>208</sup> However, how KRAP functions in the IP<sub>3</sub>R-mediated Ca<sup>2+</sup> signaling in those cancer cell lines and *in vivo* remains unclear.

In non-cancer cells, IP<sub>3</sub>Rs are directly regulated by or interact with cytoskeletal proteins,<sup>209</sup> including but not limited to Factin,<sup>210,211</sup> protein 4.1N,<sup>212</sup> myosin II,<sup>213,214</sup> ankyrins,<sup>215-219</sup> and microtubules.<sup>220</sup> This evidence suggests that IP<sub>3</sub>Rs might be responsive to mechanical microenvironments via the cytoskeleton, and further influence the intracellular Ca<sup>2+</sup> signaling. Indeed, in HMSCs, IP<sub>3</sub>R-regulated ER Ca<sup>2+</sup> release in response to optical tweezer traction is dependent on cytoskeletal structure and actomyosin contractility but not IP<sub>3</sub> level.<sup>158</sup> Moreover, in human cervical carcinoma cells, IP<sub>3</sub>Rs release ER Ca<sup>2+</sup> in response to ER-NE membrane tension, which further reinforces cortical actomyosin contractility to facilitate cancer cell transmigration through 3D collagen lattices and synthetic pores.87 These data suggest that in cancer cells, mechanical stimuli hold the potential to activate IP<sub>3</sub>Rs via cytoskeletal proteins and/or ER membrane tension to further induce Ca<sup>2+</sup> signals.

3.3.4 PLC/PIP<sub>2</sub> and cytoskeletal proteins. PLC and phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) act as upstream effectors in the Gq-PLC-IP<sub>3</sub>R pathway to activate IP<sub>3</sub>Rs and trigger ER Ca<sup>2+</sup> release.<sup>153,203,204</sup> In cancer cells, several members of the PLC family regulate the actin cytoskeleton.<sup>221-223</sup> In gastric cancer cells, PLCD1 expression reduces actin protrusion at the leading edge and inactivates cytoskeletal reorganization regulator cofilin, resulting in rounded morphology and suppressed migration in vitro and inhibited metastasis in vivo.<sup>221</sup> In highly metastatic breast cancer cells, upregulated PLCB1 cleaves PIP<sub>2</sub> at the plasma membrane to release inactivated cofilin and remodel actin cytoskeleton, therefore promoting cell migration and invasion.<sup>222</sup> In MDA-MB-231 cells, downregulation of PLCy1 impairs induced Rac1 activation and decreases actin-cytoskeleton-mediated membrane ruffles, inhibiting cell migration and invasion in vitro and lung metastasis in vivo.<sup>223</sup> In non-cancer cells, PLC/IP<sub>3</sub>R Ca<sup>2+</sup> signaling is regulated by cytoskeletal proteins,<sup>197,224,225</sup> such as F-actin<sup>197,224</sup> and filamin.225

 $PIP_2$ , which produces  $IP_3$  following PLC cleavage, regulates actin-binding proteins including talin, gelsolin, ERM proteins (ezrin/radixin/moesin), formin, and actin-related protein 2/3 (ARP2/3) to mediate actin cytoskeleton dynamics.<sup>226–228</sup> A myriad of actin-binding proteins interact with  $PIP_2$ ,<sup>229</sup> including ERM proteins and myosin I, $^{230}$  talin, $^{228}$  formins and ARP2/3, $^{227}$  and Coronin 1A. $^{231}$ 

**3.3.5 SOCE regulators and cytoskeletal proteins.** In human prostate cancer epithelial LNCaP cells, calyculin A (CalA)-caused cortical F-actin polymerization attenuates thapsigargin (TG)-induced SOCE without altering the expression level of SOCE regulators: Orai1, STIM, and transient receptor potential canonical 1 (TRPC1).<sup>232</sup> The dissociation of F-actin by Cytochalasin D (CytD) restores TG-induced SOCE in neuroendocrine differentiated LNCaP cells. The same group also reported that in LNCaP cells, cortical actin polymerization by CalA or jaspla-kinolide prevents SOCE triggered by active IP<sub>3</sub>-induced ER Ca<sup>2+</sup> depletion, while depolymerizing actin by CytD shows no effect on IP<sub>3</sub>-induced SOCE.<sup>233</sup> However, TG-induced SOCE, by inhibiting sarco/endoplasmic reticulum Ca<sup>2+</sup> ATPase (SERCA) and passively depleting ER Ca<sup>2+</sup>, are not affected by either polymerization or depolymerization of cortical actin.

In summary, from our perspective,  $Ca^{2+}$  signals are instrumental at different stages of cancer progression. Various mechanical stimuli activate intra- and inter-cellular  $Ca^{2+}$ signaling pathways in cancer cells *via* mechanosensitive channels or through crosstalk with mechanotransduction pathways. Next, we introduce the functional roles and mechanisms of another mechanosensitive biochemical effector in cancer: YAP.

# 4. Yes-associated protein (YAP) in cancer

#### 4.1 Significance of YAP in tumor progression

(Yes-associated protein) and TAZ (Transcriptional YAP co-activator with PDZ-binding motif) are two transcriptional co-activators in the Hippo pathway,  $^{234,235}$  and share ~60% similarity in protein sequences.<sup>236</sup> Binding with transcription factors in the nucleus, including YAP-TEA domains (TEADs), runt-related transcription factors (RUNXs), and p73, etc., YAP/ TAZ regulate the transcription of genes including CTGF, IGFBP3, ITGB2, BIRC5, GLI2, and AXL, etc., 234 and regulate cell fates, functions (stemness, proliferation, apoptosis, migration, etc.), organ size, and homeostasis.234,237 Shuttling between nucleus and cytoplasm is an essential characteristic of YAP/ TAZ because they only function when activated in the nucleus.90 The aberrant expression and nuclear accumulation of YAP/TAZ correlate with different cancers and at diverse tumour stages.90

Recent studies show that, in response to biophysical signals, YAP/TAZ regulate the behaviors of both cancer cells and cancerassociated fibroblasts (CAFs) during tumor initiation, growth, and metastasis. For example, a stiff substrate (40 kPa) is needed when receptor tyrosine kinase (RTK)-Ras oncogenes transform normal cells into cancer cells through a YAP/TAZ-dependent mechano-transduction pathway.<sup>238</sup> In CAFs, YAP activity is required to bridge biophysical signals from stretchable substrate and initiation of cytoskeleton remodeling, forming a self-reinforcing feed-forward loop.<sup>239</sup> This important loop maintains CAFs' phenotype and promotes tumor tissue stiffening, cancer cell growth, and invasion.<sup>239</sup>

In this review paper, we focus on the mechanobiology of YAP in cancer cells and their normal counterparts (Fig. 4). The fundamental biology of YAP/TAZ have recently been reviewed.<sup>90,94,234,240,241</sup>

#### 4.2 Biophysical stimuli induce YAP responses

In both healthy and cancer cells, YAP and TAZ proteins respond to a broad range of biophysical stimuli, such as ECM mechanics (substrate stiffness and its heterogeneous patterns; material-type; dimensionality; geometry; topology; fiber directionality; and surface porosity), cellular mechanical states (cell spreading area; focal adhesion area; cytoskeleton tension or prestress; nuclear deformation; and cell shape), cell density, and extracellular mechanical stimuli (stretch; compression; pressure; and shear). Instructed by these biophysical stimuli, YAP responds differentially, represented by its translocation between the cell nucleus (N) and the cytoplasm (C), and translates the biophysical information into cell-specific transcriptional programmes. However, upon receiving the same biophysical stimuli or being in the same mechanical state, normal cells and cancer cells show distinct responses. For example, in normal cells, most studies show a positive correlation between the YAP nucleus/cytoplasm (N/C) ratio and cell spread area; a more spread cell shows a higher concentration of YAP in the nucleus and hence a higher N/C ratio,96,98,242 resulting in a higher proliferation rate.<sup>98</sup> In contrast, in human breast cancer cells, YAP N/C ratio shows no notable correlation with cell spread area.243

Conventional studies suggest that, prevailing in the evolutionarily conserved Hippo pathway, YAP and TAZ are regulated by biochemical cues and function in the nucleus to regulate cell fate and tissue homeostasis.<sup>237</sup> Importantly, recent studies show that, in addition to biochemical cues, biophysical cues can independently regulate YAP's translocation from the cytoplasm to the nucleus through either the Hippo-dependent or -independent pathway. In the Hippo pathway, YAP/TAZ are phosphorylated by mammalian Ste20-like kinases 1/2 (MST1/ 2) and large tumor suppressor 1/2 (LATS1/2) and bind with the 14-3-3 protein and are retained in the cytoplasm.<sup>234</sup> In both normal and cancer cells, substrate stiffness can regulate intracellular distribution of YAP through Hippo-dependent mechanisms.<sup>244,245</sup> In the Hippo-independent case, extracellular biophysical cues regulate YAP translocation and bypass the Hippo pathway. For example, in the YAP-mutant cells that do not have Hippo-pathway-required interactions between YAP and LATS 1/2, substrate stiffness enhances nuclear YAP activity.<sup>98</sup> Further, a study finds that modulation of biophysical cues can even dictate the Hippo pathway in regulating the YAP translocation.98

Importantly, emerging evidence suggests that, among all the mechano-sensitive components that participate in the regulation of YAP translocation, the nucleus can serve as a previously under-appreciated mechano-sensor that directly reads and translates biophysical cues into biochemical activities that regulate YAP translocation.<sup>96,97,246</sup> However, the detailed molecular

mechanism of how biophysical cues/states trigger, regulate, and maintain YAP translocation remains unclear at this time. Consequently, the potential mechanisms underpinning nuclear mechano-regulation remain an active area of research.

**4.2.1 ECM stiffness.** In normal cells, such as human mammary epithelial cells, mouse embryonic fibroblast cells, and NIH 3T3 cells, *etc.*, that are cultured on 2D/3D environments, YAP N/C ratio shows a monotonic and positive correlation with ECM stiffness,<sup>96,98</sup> with one exception.<sup>246</sup> On 2D substrates:

(1) In mammary epithelial cells and mouse embryonic fibroblasts (MEFs), YAP N/C ratio positively correlates with substrate stiffness.<sup>96,98</sup> Cytoskeleton tension is necessary for substrate stiffness to regulate the translocation of YAP.<sup>98</sup> The force-induced enlargement in nuclear pore size has been hypothesized to facilitate YAP nuclear translocation and is actively studied now.<sup>96</sup>

(2) Recent research on NIH 3T3 cells shows no correlation between YAP N/C ratio and substrate stiffness.<sup>246</sup> Instead, YAP N/C ratio positively correlates with cellular traction force and nuclear deformation.<sup>246</sup> The data suggest that while nuclear translocation of YAP, in most normal cells, positively correlates with substrate stiffness, substrate stiffness often leads to changes in downstream cellular behaviors such as cell spread area, traction, and nuclear shapes. It is these changes that directly regulate YAP translocation, rather than the substrate stiffness itself. We hypothesize that, in this experiment, substrate stiffness does not necessarily determine contractility. Therefore, if YAP translocation is regulated by contractility, the YAP N/C ratio shows correlation with contractility instead of substrate stiffness. To decouple these behaviors and reveal the true mechanotransduction mechanisms underlying YAP translocation, more studies are required.

In 3D culture milieu, positive correlation is observed between YAP N/C ratio and environmental stiffness.<sup>247,248</sup> Importantly, in human liver organoids, signaling of integrinmediated Src family kinases (SFKs) promotes YAP activity on stiff (1.3kPa vs. 0.3 kPa) 3D matrices.<sup>248</sup> In contrast with 2D substrate findings, which suggest that YAP's response to extracellular biophysical stimuli necessitates cytoskeletal contractility,<sup>96,97,249</sup> this alternative integrin-mediated SFK mechanism insinuates that YAP is not regulated through the conventional cytoskeletal tension or the downstream nuclear mechanosensing in 3D substrates and *in vivo*. This possibility is supported by a recent finding which suggests that geometrical changes (including wrinkling) on nuclear envelopes in 2D/ 3D-cultured cells trigger diverse mechanisms to regulate YAP translocation.<sup>247</sup>

In this light, we hypothesize that a threshold of substrate stiffness may exist to determine the role of cytoskeletal tension in the regulation of YAP translocation. One recent report supports our hypothesis and shows that, in MEF cells, the unfolding of Talin by cytoskeletal tension and YAP nuclear translocation only occurs once substrate stiffness is larger than 5 kPa.<sup>250</sup> Although the two results reported in human liver organoids<sup>248</sup> and in MEF cells<sup>250</sup> are obtained from different cell types on distinct 2D/3D substrates, we reason that the



**Fig. 4** Biophysical cues regulate YAP translocation. (A) Soft substrate inhibits nuclear translocation of YAP through ILK/Merlin signalling.<sup>254</sup> Stiff substrate inhibits Merlin function and enlarges nuclear pore size to facilitate nuclear translocation of YAP.<sup>96,254</sup> (B) Cell spread area mediates RhoA-ROCK-Cofilin signalling to regulate actin polymerization and tension and translocation of YAP.<sup>98</sup> (C) Cell-PDMS contact (confined micro-fluidic channel) induces compression on cells and inhibits nuclear translocation of YAP.<sup>265</sup> Cell-cell contact increases actin belt tension and releases Merlin from gap junction to inhibit nuclear translocation of YAP.<sup>256</sup> (D) LINC- complex-dependent actin-cap and peri-nuclear force facilitates nuclear translocation of YAP.<sup>257</sup> (E) Horizontal compression of cells inhibits nuclear translocation of YAP. Horizontal stretching of cells enlarges nuclear pore size to facilitate nuclear translocation of YAP.<sup>96</sup> (F) Local compression of cell nucleus by AFM probe enlarges nuclear pore size to facilitate nuclear translocation of YAP.<sup>96</sup> Global compression of the cells by PDMS plate deactivates RhoA and triggers depolymerization of actin to inhibit nuclear translocation of YAP. The scale bar in (A) represents 5 μm length and applies to all other subfigures.

stiffness range within the organoid (1.3 kPa and 0.3 kPa) may not reach the hypothesized stiffness threshold (such as 5 kPa)<sup>250</sup> to trigger the regulatory effects of cytoskeletal tension on YAP translocation. Our hypothesis can be evaluated by targeted disruption of the actin cytoskeleton, systematic characterization of YAP translocation, and real-time measurement of nuclear envelope geometry and tension in cells experiencing a range of environmental stiffness.

In certain cancer cell lines (pancreatic, brain, and liver), YAP N/C ratio is observed to show positive correlation with substrate stiffness,<sup>245,251–253</sup> despite a few exceptions:<sup>99,254</sup>

(1) Brain cancer cells (on 10%- and 3%-acrylamide polyacrylamide (PAA) gels) and pancreatic cancer cells (on 1, 4, and 25 kPa PAA gel) show higher nucleus YAP localization on stiffer substrates.<sup>252,253</sup> Human liver cancer cells show higher nucleus YAP localization on stiffer PAA gels (1.1 kPa vs. 400 Pa).<sup>245</sup> Mechanistically, agrin and integrin sense the stiffness signals and trigger YAP translocation through two subsequent mechanisms including (a) the formation of actin stress fibers and (b) the diminishment of the Merlin function (Note: Merlin retains YAP in the cytoplasm through activation of the Hippopathway).<sup>245</sup> Although cellular traction is not measured in this study, the observed formation of actin stress fibers, induced by agrin stiffness-sensing and nuclear translocation of YAP, shows the regulatory role of cytoskeletal tension on YAP translocation. In liver cancer cells, increased substrate stiffness triggers the increase in the expression of mechano-transducer C-X-C Motif Chemokine Receptor 4 (CXCR4) and maintains the positive correlation between YAP N/C ratio and substrate stiffness.<sup>251</sup> These studies on liver cancer cells suggest that, even for the same cell type, multiple molecular pathways may co-regulate YAP translocation in response to ECM stiffness.

(2) Interestingly, in breast cancer cells, biphasic correlation is uncovered between YAP N/C ratio and substrate stiffness. YAP N/C ratio is lower than 1 on both soft (10 kPa) and stiff (57 kPa) PAA gel, while it is larger than 1 on intermediate stiff (38 kPa) PAA gel. Mechanistic studies suggest this translocation is regulated by Integrin Linked Kinase (ILK)/Merlincontrolled YAP nuclear transportation (Fig. 4A and Table 2).<sup>254</sup> Specifically, ILK locates between integrin and actin and mediates the phosphorylation of Merlin to regulate YAP translocation. Supported by a series of functional results, the study concludes that the biphasic correlation between ILK and substrate stiffness causes the highest YAP nuclear translocation on substrates of intermediate stiffness. The mechanism by which ILK expression level is regulated by substrate stiffness and causes the YAP translocation is now under investigation.

**4.2.2** Cell volume and area. In normal cells, YAP N/C ratio is positively correlated with cell spread area and volume,<sup>96,98,242,255</sup> despite one exception<sup>246</sup> (Fig. 4B and Table 2). This inconsistency may occur because cell spread area and cell volume regulate diverse downstream cell behaviors such as traction and nuclear deformation,<sup>96</sup> and these downstream behaviors can influence YAP. To identify the ground-truth regulators of YAP translocation, these cell behaviors, along with cytoskeletal

tension and nuclear deformation, need to be investigated in a decoupled fashion.

In cancer cells, only one study has been conducted and shows no correlation between YAP N/C ratio and cell size.<sup>243</sup> This study focused on single metastatic breast cancer cells (MDA-MB-231) and metastatic D3H2LN cells harvested from mouse lymph nodes in MDA-MB-231-injected mice.<sup>243</sup> Further research on other cancer cell types needs to be conducted to verify if YAP is not correlated with cell area in all cancer cells.

4.2.3 Cell density. Regardless of the sizes of multicellular structures, YAP N/C ratio consistently shows negative correlation with cell density. Compared to single cells, the regulatory mechanism of YAP translocation in multicellular structures is different due to the existence of cell-cell contacts but is still related to cytoskeletal tension. In normal cells, YAP N/C ratio shows negative correlation with cell density, and more actin belts are observed in denser cells (Fig. 4C and Table 2).98,256 By manipulating the formation and tension of the actin belt, this study shows that, at high cell density, increased tension within the actin belt disassociates Merlin from adhesion junctions and facilitates YAP cytoplasmic retention in a Hippodependent way.<sup>256</sup> In particular, this result indicates the importance of cortical actin structures in cell-cell contact, contrasting with the result suggesting that periphery actin structures have no regulatory effect on YAP translocation in single cells.<sup>257</sup>

In another study on brain cancer cell lines, the YAP N/C ratio is negatively correlated with cell density.<sup>253</sup> Merlin expression shows a positive correlation with cell density, implying that cell density regulates YAP intracellular distribution through Hippo-dependent mechanisms in cancer cells. The potential roles of cytoskeletal structure and tension in cancer cells must be further investigated.

4.2.4 Cytoskeleton tension and cell contractility. The cytoskeleton consists of three main components: actin, microtubule and intermediate filaments.<sup>31</sup> Actin filament is the main tension-bearing structure. Most research found a functional relationship between nuclear YAP accumulation and actin tension.<sup>96,97</sup> Inferred by the cell traction, cytoskeletal tension is one of the essential downstream parameters regulated by substrate stiffness and cell spreading area. These four characters positively correlate with each other.15,258,259 In NIH 3T3 normal cells, cell traction positively correlates with YAP N/C ratio.<sup>246</sup> In mesenchymal stem cells (MSCs), reduced traction, which is induced by the inhibition of myosin II or ROCK, correlates with reduced YAP N/C ratio.97 Shown in normal MEF cells, high substrate stiffness couples with high cell traction and high YAP N/C ratio.96 Following depolymerizing actin by cytochalasin D, YAP N/C ratio shows no correlation with substrate stiffness.96

In the same type of MEF cells, perinuclear traction force and actin-cap are observed for the first time. Disrupting the linker of nucleoskeleton and cytoskeleton (LINC) complex (a transmembrane protein complex that locates on the nuclear envelope and connects the nuclear interior with the cytoskeleton) reduces perinuclear force, eliminates actin-cap formation, and shows the reduced YAP N/C ratio without influencing cell

Table 2 YAP translocation induced by mechanical stimu	มในร
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Mechanical stimulus	Normal/ cancer	Cell type	Relation with nuclear translocation	Proposed mechanism or related protein	Ref
Substrate Stiffness	Normal	Mammary epithelial cells	Positive	F-Actin-capping/severing proteins	98
	Cancer	MEF NIH 3T3 cells Pancreatic cancer cell Liver cancer cell	Positive No correlation Positive Positive	Cofilin, CapZ, and Gelsolin Nuclear pore size increase N/A N/A Agrin/integrin mediated stiffness	96 246 252 245
		Liver cancer cell	Positive	sensing and formation of stress fiber CXCR4 mediated YAP cytoplasmic degradation	251
		Brain cancer cell, IOMM-Lee,	Positive	Merlin mediated YAP	253
		Tumor repopulating cells (ovarian cancer cell line A2780, human MCF-7 breast cancer cell line and	Positive (but on different substrate type)	Cdc42-mediated F-actin and Lats1 interactions	406
		Breast cancer cell	Biphasic	ILK and Merlin mediated	254
Cell area	Normal	Mammary epithelial cells	Positive	F-Actin-capping/severing proteins Cofilin, CapZ, and Gelsolin	98
		MSC	Positive	Rho/ROCK mediated actin	242
		NIH 3T3 cells	$(> \sim 1000 \ \mu m^2)$ not related, $(< \sim 1000 \ \mu m^2)$ YAP in cytoplasm	Nuclear deformation	246
	Cancer	Breast cancer (MDA-MB-231)	No correlation	N/A	246
Cell traction	Normal	MEF	Positive	Nuclear pore size change	96
		NIH 3T3 cells	Positive (not decoupled with nucleus deformation)	Nuclear deformation	246
			No correlation (decoupled with nucleus deformation)	N/A	
		Mesenchymal Stem Cells	Positive (when decrease traction)	Nuclear deformation	97
Perinuclear Traction	Normal	MEF	Positive	Actin-cap	257
Peri-cell traction	Normal	MEF	No correlation	N/A	257
		normal cell (MDCKII)	Negative	Actin belt mediated Merlin	256
Fluid shear stress	Normal	zebrafish endothelial cells and human pulmonary artery endothelial cells	Positive	Cortical actin bundles release YAP from binding with angiomotin	260
	Cancer	Human prostate cancer cells	Positive	Polymerization of F-actin	261
Stretch	Normal	MEF	Positive (cyclic)	N/Å	263
		Mesenchymal stem cells	Positive	Nuclear deformation	97
Compression	Cancer	cervical cancer cell	Negative	F actin depolymerization and RhoA deactivation	264
		Human fibrosarcoma HT1080	Negative	Ca <sup>2+</sup> dependent	266
		Osteosarcoma, U2OS	Negative	N/A	265
	Normal	MCF-10A	Negative	F actin depolymerization and RhoA deactivation	264
		MEF	Positive	Nuclear pore size increase	96

periphery traction (Fig. 4D and Table 2).<sup>257</sup> This finding suggests that the perinuclear cytoskeletal tension and structure enable transmitting the force into the nucleus to regulate YAP translocation.

In the presence of cell-cell contact, the cortical actin tension regulates the translocation of YAP in a Hippo-dependent way. In normal MDCKII cells, increased actin belt tension (reflected by the amount of colocalized myosin-II and F-actin) negatively regulates the YAP N/C ratio by releasing Merlin from the adhesion junction to enhance the retention of YAP in the cytoplasm.<sup>256</sup>

Overall, in normal cells, YAP N/C ratio positively correlates with overall cell traction. However, the intracellular distribution of cytoskeletal tension, *i.e.*, in the perinuclear and cell periphery regions, may have differential regulatory roles on YAP translocation. Additionally, cytoskeletal tension/structure and the LINC complex are needed for cells to sense extracellular biophysical stimuli and subsequently trigger YAP translocation. Third, nuclear deformation is positively correlated with YAP N/C ratio and traction force<sup>96,255</sup> and needs to be decoupled from traction to determine if it is an independent regulatory effector. Fourth, unlike in normal cells, the correlation between cell traction and YAP translocation in cancer cells is still lacking.

**4.2.5** Nuclear mechanics. In normal cells, the extent of nucleus deformation, *e.g.*, flattening, shows a positive correlation with cell overall traction, substrate stiffness, and YAP accumulation in the nucleus.<sup>96,97</sup> In MEF cells, the disruption of the LINC complex—by blocking the interactions between

Nesprin located at the outer nuclear membrane, connecting the cytoskeleton with Sad1p-UNC-84 (SUN) proteins located at the inner nuclear membrane, and connecting Nesprin with the nucleoskeleton-does not affect cell traction force but reduces both nucleus deformation and YAP N/C ratio. It suggests that YAP translocation is regulated by nuclear mechano-sensing, potentially through geometrical change, membrane tension or potential mechano-sensing within the LINC complex, induced by the cytoskeletal tension. When the apical surface of the cell is compressed by atomic force microscopy (AFM) tips outside the nucleus (*i.e.*, only at the cytoplasm and not compressing the nucleus), cells show no nuclear deformation and YAP shows no nuclear translocation (Fig. 4F and Table 2). In contrast, when the apical surface above the cell nucleus is compressed by AFM tips following the cytoskeleton disruption, YAP shows nuclear translocation along with nuclear deformation.96 This finding indicates that, in YAP regulation, the nucleus can function as a mechano-sensor independent of cytoskeletal tension and force transmission into the nucleus is necessary to trigger YAP translocation. Further, nuclear pore size shows a positive correlation with nuclear flattening and YAP nuclear translocation, raising the possibility that an increase in nuclear pore size-induced by nuclear flattening-is likely to regulate nuclear translocation of YAP.<sup>96</sup> However, in this study, nuclear deformation and the force that is transmitted into the nucleus are not decoupled.

To decouple the roles of nuclear deformation and cytoskeletal tension in YAP regulation under stretching, another study employed two drugs with distinct functions: ML7 and Y27632. ML7 is an inhibitor of myosin-II b and reduces the cytoskeletal tension but keeps the stress fibers and nuclear deformation. In the cells under cyclic stretching treated by ML7, YAP shows nuclear translocation. In contrast, Y27632 is an inhibitor of ROCK and eliminates the cytoskeletal tension as well as nuclear deformation. Cells treated by Y27632 under cyclic stretching show no YAP translocation into the nucleus.97 These results indicate that cytoskeletal contractility is not necessary in regulating YAP translocation. Instead, the force sensed by the nucleus is required to regulate YAP translocation. A recent study corroborates this indication. The study changes the nuclear stiffness through an up-regulation of Lamin A expression and observes that YAP N/C ratio correlates with nuclear deformation but not traction force.246 These results suggest that YAP translocation is regulated by nuclear deformation but not necessarily by the force transmitted through the LINC complex. However, Lamin A not only affects nuclear stiffness but also serves as the structural component that is downstream of the LINC complex and might affect the potential nuclear mechano-sensing through this route. Hence, how nuclear mechano-sensing regulates YAP translocation at the precise molecular level needs to be further investigated.

To address this question, we propose three potential approaches. First, one can achieve similar nuclear deformation in cells by different force transmission methods including stretching and compression and measuring the corresponding difference in YAP translocation. If the nuclear geometry regulates YAP translocation, then the YAP N/C ratio should be similar in cells that experience similar nuclear deformation regardless of the types of forces applied. Second, we can disrupt the cytoskeleton and directly apply force on the nucleus through either the LINC complex or other protein complexes, potentially with magnetic beads, followed by observing the differential relationship between YAP translocation and nuclear deformation. If force transmission through the LINC complex regulates YAP translocation, then the YAP N/C ratio should increase noticeably when forces are applied via the LINC complex but not via other protein complexes. Third, one can maintain the level of nuclear deformation without interfering with the nuclear force transmission using methods such as keeping Lamin A expression constant and stretching cells to increase the force transmitted into the nucleus through the LINC complex. If the nuclear deformation regulates YAP translocation, the YAP N/C ratio should remain stationary regardless of the magnitude of force transmitted into the nucleus. These strategies enable the decoupling of nuclear deformation from the force transmitted into the nucleus (through and not through LINC) and bring us closer to the discovery of the molecular underpins in YAP translocation.

Next, we discuss how YAP translocation responds to actively applied extracellular force.

4.2.6 Fluid shear stress. Fluid shear stress induces nuclear translocation of YAP in both normal and cancer cells but through different mechanisms (Table 2).<sup>260,261</sup> In zebrafish endothelial cells and human pulmonary artery endothelial cells, shear stress (15 dynes  $cm^{-2}$  for 10 min) facilitates the formation of cortical actin bundles and release YAP from binding with angiomotin to trigger the nuclear translocation of YAP, independent of Hippo pathway.<sup>260</sup> In this process, nuclear mechano-sensing is not required. In human prostate cancer cells, shear stress (0.05 dyne cm<sup>2</sup> for 6 h) facilitates the polymerization of F-actin through ROCK-LIMK-cofilin signaling and triggers the nuclear translocation of YAP.<sup>261</sup> In hepatocellular carcinomas, fluid shear stress (1.4 dyne cm<sup>2</sup> for 2–8 h) triggers the nuclear YAP translocation in a F-actin-dependent way.<sup>262</sup> Whether nuclear mechano-sensing and cortical actin tension are involved in this mechanism remains unclear.

**4.2.7 Tension and compression forces.** In Section 4.2.4, we show that the cytoskeleton tension transmits into the nucleus to regulate YAP translocation. In parallel, external forces that are actively applied on cells also regulate YAP translocation, in two potential ways: (1) activate mechano-sensors on the cell membrane to trigger downstream YAP-related signaling; and (2) trigger nuclear mechano-sensing.

In normal cells, both static and cyclic stretching trigger nuclear translocation of YAP (Fig. 4E and Table 2).<sup>97,98,263</sup> In MEFs, static stretching of the cell monolayer induces increased YAP N/C ratio.<sup>98</sup> In MSCs, when the cytoskeletal contractility is inhibited by ML7 but the actin stress fibers are maintained, cyclic stretching can cause nuclear deformation and YAP nuclear translocation.<sup>97</sup>

Active compression on cells does not cause a universal trend on the regulation of YAP translocation. Compression force

#### Review

(1.5 nN), applied by AFM tips on the normal and cytoskeletondisrupted MEF cells at the apical surface above the nucleus, triggers YAP nuclear translocation.96 In both HeLa (cervical cancer cell line) and MCF-10A (normal mammary epithelial cell line), compression (24 Pa) applied by a polydimethylsiloxane (PDMS) sheet causes F-actin depolymerization and YAP translocation into the cytoplasm (Fig. 4F).<sup>264</sup> Similar to preceding research, deformations of the cell and the nucleus are not quantified in this study. In the osteosarcoma line, cells under narrow confinement from micro-fluidic devices show YAP cytoplasm translocation. However, the cells on the line patterns (width range: 5-50 µm) without confinement show no YAP translocation, even with large nucleus aspect ratio.<sup>265</sup> This result implies that (1) the aspect ratio of the nucleus does not regulate YAP translocation, and (2) the real regulatory parameter of YAP translocation is influenced by force transmitted into the nucleus, instead of nuclear geometry. Compression on human fibrosarcoma cells inhibits RhoA activity through TRPV4 mediated Ca<sup>2+</sup> currents and cause the cytoplasmic translocation of YAP.<sup>266</sup>

#### 4.3 Summary of YAP mechano-transduction

The key understandings of the roles of YAP in mechanotransduction and the direct regulators of YAP are:

(1) YAP acts as a mechano-transducer that transmits the extra- and intra-cellular biophysical cues into the cell nucleus and regulates cell functions through binding with transcription factors.

(2) YAP itself is unlikely to be a direct mechano-sensor that senses the biophysical cues. The mechano-sensors, such as integrin and potentially the cell nucleus, convert biophysical cues into chemical signals that are transmitted by YAP activation.

(3) Mechanistically, the mechano-regulation of YAP is believed to be mainly through the F-actin cytoskeletal tension and nuclear envelope mechanics.

(4) The nucleus is a promising mechano-sensor that can directly sense the biophysical signals.

How the nucleus senses the force and regulates YAP is being actively studied. Elosegui-Artola proposes that the size changes in nuclear pores, induced by nucleus flattening, regulate YAP translocation.<sup>96</sup> However, because of the challenges in manipulating the size of nuclear pores in a controlled manner, this hypothesis is still under active investigation. In line with the finding that the nucleus is a direct mechano-sensor, we hypothesize that the combination of the LINC complex and nucleoskeleton may function as an alternative route to transmit force and regulate YAP. Our hypothesis is supported by a recent study that shows that, in the nucleus isolated out of the cell body, force transmission through the LINC complex and nonspecific bindings into the nucleus triggers distinct changes in nuclear stiffness. Since the size changes in nuclear pores are unlikely to affect nuclear stiffness, we hypothesize that certain other mechano-sensitive underpins within the LINC complex and nucleoskeleton may respond to the force transmitted into the nucleus and alter nuclear mechanical states.

If the mechanisms of mechano-transduction through YAP are clear, it offers new opportunities to develop mechanomedicine for cancer treatment because of the important role of YAP in maining mechanical homeostasis.<sup>94</sup> We propose:

(1) To reduce the possibility of tumor initiation in stiffened tissue, we can inhibit the stiffness sensing in normal cells through YAP translocation since YAP is required for RTK-Ras oncogenes to transform normal cells into tumor cells on stiff ECM.<sup>238</sup> If the mechano-transduction through YAP is inhibited in normal cells within fibrosis tissue, which has higher stiffness and higher possibility for tumor initiation, the transformation of normal cells can be suppressed.

(2) To reduce tumor tissue stiffness and cancer cell extravasation, we can inhibit YAP-mediated mechano-transduction in CAFs because YAP activity is required for CAFs-dependent matrix stiffening, cell invasion, and angiogenesis.<sup>239</sup>

### 5. MicroRNA in cancer

#### 5.1 miRNA biogenesis

MicroRNAs (miRNAs) are ~22 nucleotide (nt) RNA, first discovered in *Caenorhabditis elegans* in 1993.<sup>267</sup> Under most conditions, miRNAs interact with the 3' untranslated region (UTR) of target messenger RNAs (mRNAs) to cause mRNA deadenylation and decapping as well as to attenuate the translational output.<sup>268,269</sup> In addition, multiple reports have demonstrated the capability of miRNAs to target protein-coding sequences (CDS) and 5' UTR.<sup>270-272</sup> The miRNA biogenesis can be classified into canonical and non-canonical pathways.

In the canonical pathway, miRNAs are transcribed by RNA polymerase II (Pol II) (Fig. 5A). The Pol II-transcribed primary (pri-) miRNAs are capped and polyadenylated, harboring one or multiple hairpin structure(s), which contain the miRNA sequence.<sup>273,274</sup> Processing of the pri-miRNAs is carried out in the nucleus by a heterotrimeric complex, Microprocessor, comprised of one molecule of the RNase III enzyme Drosha and two molecules of Digeorge critical region 8 (DGCR8; named Pasha in flies and nematodes).<sup>275-280</sup> Drosha possesses two RNase III domains that each cleaves one strand of the stem in the pri-miRNA hairpin, which liberates a 60 nt to 70 nt stemloop called a precursor (pre-) miRNA with a characteristic 3' hydroxyl group (OH), overhangs of 2 nts, and a 5' phosphate (P). The generated pre-miRNAs are exported to the cytoplasm by an exportin 5 (XPO5)/RanGTP complex and processed by another RNase III enzyme Dicer.281 As an endonuclease with two RNase III domains, Dicer functions in concert with trans-activationresponsive RNA-binding protein (named TRBP in mammals and Loquacious in flies).<sup>282</sup> In this process, Dicer releases a dsRNA that is  $\sim 22$  base-pairs long from the stem of the premiRNA to the cleavage site contiguous to the apical loop and creates a mature miRNA duplex that interacts with the Argonaute (AGO) proteins.<sup>283,284</sup> Afterwards, the AGO unwinds the RNA duplex and promotes the expulsion of the passenger strand to form the mature RNA-induced silencing complex (RISC).<sup>285</sup> Depending on the origin from the hairpin arms,



**Fig. 5** miRNA biogenesis pathway. (A) Canonical miRNA biogenesis pathway. In the nucleus, primary (pri-) miRNAs are transcribed by Pol II and then processed by the Microprocessor complex containing one Drosha and two DGCR8 to form pre-miRNAs.<sup>273</sup> Pre-miRNAs are exported to the cytoplasm by the complex of XPO5/RanGTP. Subsequently, pre-miRNAs are cleaved by Dicer to form ~22 nucleotides miRNA duplex. miRNA guide strand is then loaded into the AGO to form the RNA-induced silencing complex (RISC), the passenger strand is degraded. Drosha-independent miRNA biogenesis pathways. In the mirtron pathway, pre-miRNAs are spliced and debranched from the intron region of transcript, which bypass Drosha processing. After that, the intron-derived pre-miRNA access the canonical miRNA pathway.<sup>293-295</sup> In the transcription start site (TSS) miRNA biogenesis pathway, the 5' end of the pre-miRNA hairpin intermediate contains an 7-methylguanosine (m<sup>7</sup>G)-Cap and the 5' end of the pre-miRNA hairpin intermediate contains an 7-methylguanosine (m<sup>7</sup>G)-Cap and the 5' end of the pre-miRNA hairpin intermediate contains an 7-methylguanosine (m<sup>7</sup>G)-Cap and the 5' end of the pre-miRNA hairpin generated by transcription initiation directly, and the 3' end generated by transcription termination. The Capped pre-miRNA are exported by XPO1 protein and bypass Drosha processing. The 3p-capped miRNA is loaded onto the AGO complex, but the 5p-capped miRNA is degraded.<sup>296</sup> Biogenesis of miR-451. pri-miR-451 is cleaved by the Microprocessor complex and bypass Dicer. Pre-miR-451 is directly loaded into AGO2, which cleaves the 3p arm of the hairpin. Poly-A specific ribonuclease (PARN) further trims the 5p arm to form miR-451.<sup>292</sup> (B) Hippo-YAP signaling pathway affects the miRNA biogenesis.<sup>380</sup> At higher cell density, translocation of nuclear YAP/TAZ into cytoplasm inhibits LIN28, upregulates let-7a and let-7b, and represses the Dicer levels.<sup>381</sup> The scale bar in (A) represents 5 µm length and applies to all other subfigures.

the mature miRNA is designated as either the 5p or the 3p miRNA. The initiated RISC then identifies a specific mRNA sequence by complementary base-pairing, resulting in translation inhibition and/or RNA degradation.<sup>286</sup>

Numerous non-canonical miRNA biogenesis pathways have been identified.<sup>287</sup> These pathways take advantage of distinct combinations of the proteins engaged in the canonical pathway, namely Drosha, Dicer, XPO5, and AGO. Readers are referred to recent in-depth reviews for more information about non-canonical miRNA biogenesis pathways.<sup>288–300</sup>

#### 5.2 Significance of miRNA regulation in cancer

During the last decade, convincing evidence has clarified that miRNA expression is dysregulated in human malignancies through diverse mechanisms, including miRNA biogenesis defect, miRNA gene mutation, and dysregulated transcriptional control or epigenetic modification of miRNA genes.<sup>301</sup>

**5.2.1 miRNA biogenesis defect.** As introduced above, miRNA biogenesis involves delicate processing by several enzymes and regulatory proteins, including Drosha, DGCR8, XPO5, Dicer and AGO.<sup>302</sup> Therefore, mutation or abnormal expression of any factor of the miRNA biogenesis machinery could trigger an aberrant expression of miRNAs.<sup>303</sup>

Microprocessor cleavage of the pri-miRNA is the initial processing step during miRNA biogenesis. Single-nucleotide substitution/deletion of the Microprocessor components Drosha and DGCR8 (15% of 534 Wilms tumors) is associated with diminished expression of mature let-7a and miR-200 family members.<sup>304</sup>

Considering the vital role of XPO5 in the nuclear export of pre-miRNAs, it is not surprising that downregulation of XPO5 causes decreased cellular proliferation, attenuated invasion, arrest of G1/S cell-cycle, and downregulation of pivotal oncogenic miRNAs (*e.g.*, miR-21, miR-10b, miR-27, miR-182 and miR-155) in colorectal cancer (CRC) cells.<sup>305</sup> Another example of dysregulation of XPO5 in cancer is that phosphorylation of XPO5 by hyper-activated ERK can repress the recruiting and exporting of pre-miRNA, which globally suppress miRNA biogenesis in hepatocellular carcinoma (HCC).<sup>306</sup>

Universal downregulation of miRNAs due to defective processing by Dicer is rising as a prevalent hallmark of cancer.<sup>307</sup> In the *DICER1* gene, somatic 'hotspot' mutations at the four catalytic residues in the RNase IIIb domain (D1709, E1705, E1813, D1810) and one catalytic residue in the RNase IIIa domain (G1809) were identified in ovarian sex cord-stromal tumors, pediatric tumors and endometrial tumors.<sup>308–310</sup> Likewise, 15 RNase IIIb hotspot in uterine corpus endometrial carcinoma (UCEC) cases show down-regulation of specific 5p miRNAs.<sup>311</sup>

AGO2, the only slicing protein in the AGO family that cleave miRNA duplexes, plays a vital role in the accumulation of mature miRNAs.<sup>312</sup> Acetylation, a novel post-translational modification (PTM) of AGO2, boosts cancer progression by specifically affecting miR-19b levels.<sup>313</sup> Additionally, the AGO2 expression levels in HCC specimens are significantly higher in comparison to adjacent non-tumor liver.<sup>314</sup>

**5.2.2 miRNA gene mutation.** Abnormal miRNA expression in malignant cells can derive from the alteration of miRNA in the genomic location and/or genomic copy number (amplification, deletion, or translocation).<sup>315</sup> The first known miRNA gene locus change is the deletion of miR-15a/16-1 cluster at chromosome 13q14, which is usually detected in B-cell chronic lymphocytic leukemia (CLL).<sup>316</sup> Loss of miR-143/145 expression is often observed in pancreatic cancers with KRAS mutations,

and restoration of these miRNAs eliminates tumorigenesis.<sup>317</sup> Myeloid-specific miR-146a deletion promotes colonic inflammation and cancer.<sup>318</sup> Mechanistically, miR-146a is pivotal for preventing colitis and colitis-associated CRC through targeting TNF receptor associated factor 6 (*TRAF6*), an IL-17R signaling intermediate, to restrict intestinal epithelial cells (IEC) responsiveness to IL-17.

Amplification of miRNA genomic loci also exists. The miR-17–92 cluster is amplified in a variety of tumors, which resulted in the upregulation of the miRNAs, thus stimulating tumor development.<sup>319</sup> Overexpression of miR-21, because of the amplification in 17q23–25, causes low expression of the tumor suppressor gene, phosphatase and tensin homolog (*PTEN*), in ovarian cancer.<sup>320</sup> In fact, the upregulation of miR-21 has been revealed in numerous cancers, which has an effect in boosting drug resistance of cancer cells.<sup>321</sup> Due to amplification of 3q26.2, a cancer-associated miRNA, miR-569, contributes to ovarian and breast cancer cell survival and proliferation.<sup>322</sup>

A high-resolution array-based assay in 227 specimens detected DNA copy number alterations in genomic loci consist of miRNA genes in ovarian cancer (37.1%), breast cancer (72.8%), and melanoma (85.9%).<sup>323</sup> Genome-wide investigations revealed that 98 of 186 (52.5%) miRNA genes are in cancer-associated genomic regions or in fragile sites.<sup>324</sup> In summary, abnormal miRNA expression in cancer cells could develop from the amplification or deletion of individual genomic regions containing the miRNA genes.

**5.2.3 Dysregulated transcriptional control of miRNA genes.** miRNA expression is closely regulated by several vital transcription factors, including *MYC* and *p53*. The activation of oncogenic transcription factor *MYC* widely affects miRNA downregulation.<sup>325</sup> *MYC* regulates the transcription of miR-17–92 cluster, which in turn maintains a tumor state by inhibiting chromatin regulatory genes *Sin3b*, *Hbp1*, *Suv420h1*, *Btg1*, and the apoptosis regulator *Bim*.<sup>326</sup> Also, *MYC* inhibits the activity of miRNA cluster let-7a-1–let-7d promoter by binding to the non-canonical E-box 3 downstream of the transcription initiation sites, while it strengthens promoter activity by binding to the canonical E-box 2 upstream of the transcription initiation sites.<sup>327</sup> Moreover, *MYC* represses the miR-15a, miR-16, miR-29a, miR-30, miR-122, miR-148a, and miR-363 by binding to their promoter in different cancer cells.<sup>328–331</sup>

In addition, the *MYC*-miRNA feedback loop is indispensable for the development of HCC. miR-122 indirectly suppresses *MYC* expression by targeting *Tfdp2* and *E2f1*. Furthermore, miR-148 directly targets the 3' UTR of *MYC* and inhibits *MYC*, while miR-363 directly targets the 3' UTR of ubiquitin-specific protease 28 (*USP28*) and indirectly destabilizes *MYC*.<sup>330</sup>

Another example is how p53 regulates miRNA abundance to exert its tumor suppressive activity.<sup>332</sup> p53 is one of the most ubiquitous tumor suppressors, whose mutation is detected in approximately 50% of human cancers.<sup>333</sup> p53 can induce the upregulation of miR-34a to prompt apoptosis, cell-cycle arrest and cell senescence through associating with the promoter of the miR-34a gene.<sup>334</sup> As a feedback loop, miR-34a inhibits p53 expression by targeting sirtuin 1 (*SIRT1*), which is a negative

regulator of *p53 via* deacetylation.<sup>335</sup> Further, the miR-34 family inhibits tumor growth and progression by targeting regulatory factors including cyclin-dependent kinase 4/6 (*CDK4/6*), cyclin E2, and anti-apoptotic protein B-cell lymphoma 2 (*BCL2*), which are engaged in cell proliferation, the cell cycle, EMT, metastasis, and stemness.<sup>336</sup> More studies revealed that *p53* regulates the expression of a range of miRNAs, such as miR-605 miR-1246, miR-143 and miR-107, to perform its function.<sup>337–339</sup>

Overall, *MYC* and *p53*, two of the most comprehensively studied transcription factors, regulate miRNA expression. Other transcription factors and miRNA co-regulatory networks, such as *E2Fs*/miR-17/20 and *PITX3*/miR-133b, have been discovered in multiple tumors.<sup>340</sup>

**5.2.4 Dysregulated epigenetic modification of miRNA genes.** Dysregulated epigenetic modifications include changes in genomic DNA methylation, as well as histone methylation and acetylation.<sup>341</sup> miRNAs inhibit epigenetic modification enzymes involved in epigenetic regulation and construct a triangle regulation "epi-miR-epi" feedback loop.<sup>342</sup> For example, the increased expression of EZH2 in patients with serine peptidase inhibitor, Kazal type-1 (SPINK1)-positive prostate cancer results in the epigenetic silencing of miRNA-338-5p/421. In contrast, the exogenous expression of miRNA-338-5p/421 in SPINK1-positive cells eliminates carcinogenic properties and exhibits lower tumor burden and distant metastasis.<sup>343</sup>

Compared with healthy individuals, the methylation level of the nine CpGs of the miR-223 promoter was significantly lower in atherosclerotic cerebral infarction (ACI) patients but higher in carotid atherosclerotic patients.<sup>344</sup> A total of seventeen miRNAs were upregulated higher than 3-fold after simultaneous treatment with DNA methylation and histone acetylation inhibitors. miR-127, one of 17 miRNAs located within a CpG island, is highly induced after treatment. Consistently, miR-127 is lowly expressed in the malignant cells, indicating that it is subject to epigenetic silencing.<sup>345</sup> Further, decreased expression of miR-152/-137 and miR-34b/c is associated with DNA hypermethylation in endometrial, lung and gastric cells, respectively.<sup>346-349</sup> The above evidence spotlights the intricate interpretation between miRNAs and the epigenetic architecture, revealing that abnormal DNA methylation and histone acetylation of miRNA genes can serve as biomarkers for cancer diagnosis and therapeutics.<sup>350</sup>

#### 5.3 Mechanosensitive miRNA in cancers

Multiple mechanosensitive miRNAs (mechanomiRs) have been identified by miRNA microarray screening of either longitudinally or transversely stretched diaphragms from mice.<sup>351</sup> Over the past few years, an increasing number of miRNAs have been reported to interact reciprocally with ECM proteins and regulate mechanotransduction *via* distinct mechanisms.<sup>352</sup> miRNAs play a role in ECM regulation by directly targeting mRNAs that encode ECM proteins or by indirectly regulating the expression of genes that modulate the synthesis/degradation of ECM proteins (Table 3). Interestingly, different miRNAs from the miR-17–92 cluster are involved in both regulatory mechanisms.

Fibronectin (Fn) is a glycoprotein found in the ECM and the generation of active Fn fibers is required for collagen I matrix assembly. The ECM network is initially constructed by depositing Fn fibers, followed by collagen I fibers, which preferentially interact with the relaxed Fn in the ECM.353,354 miR-17, a member of miR-17-92 cluster, represses the expression of Fn which leads to reduced cell adhesion, migration, and proliferation.<sup>355</sup> In addition, miR-143 can directly target the 3' UTR of Fn type III domain containing 3A (FNDC3A) and repress its expression level. Therefore, upregulated miR-143 facilitates liver tumor cell invasion and metastasis, as local liver and distant lung metastasis were significantly reduced when miR-143 expression was suppressed.<sup>356,357</sup> Another example is let-7e-5p, a member of the let-7 family, reported as mechanomiR, showing more than 1.5-fold downregulation in atrophic skeletal muscle; dysregulation of let-7e-5p may trigger muscle fibrosis by targeting the ECM proteins: Col1a1, Col1a2, Col3a1, Col24a1, Col27a1, Itga1, Itag4, Scd1, and Thbs.<sup>351</sup>

The miR-17–92 cluster can form an autoregulatory feedback loop with E2F transcription factors, thereby suppressing the expression of many tumor-associated proteins.<sup>358</sup> Induced by increased stiffness in human and mouse tissue, miR-18a from the mi-17–92 cluster, leading to reduced levels of the tumor suppressor *PTEN* by base-pairing with the 3' UTR of *PTEN*.<sup>359</sup> Increased ECM stiffness could modulate *PTEN* suppression by

Table 3	Functions of miRNA	n regulating	mechanotransduction,	mechnao-memory,	YAP, and	calcium sig	gnaling
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	microRNA	Function	Ref.
Mechanotransduction	miR-17	Repress the expression of fibronectin	355
	miR-143	Target the 3' UTR of fibronectin type III domain	357
	let-7e-5p	Trigger muscle fibrosis by targeting the ECM proteins: Col1a1, Col1a2, Col3a1, Col24a1, Col27a1, Itga1, Itag4, Scd1, and Thbs1	351
	miR-18a	Suppress PTEN via $\beta$ -catenin stimulation of MYC-driven miR-18a and HOXA9	359
Calcium signaling targeting	miR-34a	Decreased Ca <sup>2+</sup> influx	364
	miR-195	Regulate mitochondrial Ca <sup>2+</sup> uptake by downregulating MICU1	375
	miR-27a	Downregulate the ER-located Ca <sup>2+</sup> transporter CACNA2D3	369
	miR-28	Downregulate TRPM7	371
	miR-25	Downregulate MCU	374
Mechano-memory, crosstalk with YAP	miR-21	Function as a mechanical memory keeper in myofibroblast activation and fibrogenesis	392
	let-7a and	Downregulated let-7a and 7b expression rescues the miRNA biogenesis	381
	let-7b	defects observed following TAZ/YAP knockdown	
	miR-130a	Promote YAP-induced tumorigenesis and liver enlargement	386
	miR-130b	Target the MST1 and SAV1 resulting in Hippo signaling pathway inactivation	387

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directly suppressing *PTEN via*  $\beta$ -catenin stimulation of MYCdriven miR-18a and by indirectly reducing *PTEN* through the levels of homeobox A9 (HOXA9) regulation.<sup>359</sup> In breast cancer, HOXA9 directly binds to the *PTEN* promoter to regulate its expression and inhibit the malignancy.<sup>359,360</sup> *PTEN* loss in stromal fibroblasts promotes ECM deposition and alignment independently from cancer cells' presence, and this reorganization regulates cancer cell behavior.<sup>361</sup> Therefore, stromal matrix stiffness controls cellular ECM deposition through the regulation of miRNA expression.

Furthermore, 122 miRNA families with their 73 mRNA targets which encode cytoskeleton-actin-matrix (CAM) proteins were identified in endothelial cells.<sup>362</sup> The miRNA-CAM mRNA regulatory network is demonstrated to counteract the effects of ECM stiffness and promote mechanical stability of tissues.<sup>362</sup>

# 5.4 Molecular mechanism of the crosstalk between miRNA and Ca<sup>2+</sup> signaling

In Section 3, we discussed that the intracellular  $Ca^{2+}$  signaling links to almost every cancer hallmark. Emerging studies have illustrated that miRNAs play a crucial role in regulating intracellular  $Ca^{2+}$  dynamics through the SOCE pathway, calcineurin/ NFAT signaling, and  $Ca^{2+}$  ion channels (Table 3).

In T cells, SOCE is the central pathway to modulate cellular activation, proliferation, apoptosis, and migration.<sup>363</sup> In the human Jurkat T cell line, miR-34a overexpression significantly reduces calcium influx through targeting SOCE-related genes (*ITPR1, ITPR3, CALM3, ATP2A2* and *ATP2A3*) and calcineurin/NFAT signaling related genes (*RCAN1, PPP3R1* and *NFATC4*).<sup>364</sup>

miR-27a is involved in different regulatory functions in different types of cancer, and is upregulated in breast cancer,<sup>365</sup> ovarian cancer,<sup>366</sup> and prostate cancer.<sup>367</sup> In breast carcinoma, ER-located Ca<sup>2+</sup> transporter *CACNA2D3* is frequently methylated and contributes to metastasis.<sup>368</sup> In Mycobacterium tuberculosis (*Mtb*) infected peripheral blood mononuclear cells, miR-27a is abundantly expressed and contributes to autophagy inhibition through down-regulating ER Ca<sup>2+</sup> signaling by directly targeting *CACNA2D3*.<sup>369</sup> Thus, the study of miR-27a targeting *CACNA2D3* in cancer metastasis may support the development of antimetastasis therapeutic approaches.

TRPM7 forms a constitutively active Ca<sup>2+</sup> permeable channel, which regulates diverse cellular processes in healthy and tumor cells.<sup>370</sup> In glioblastomas, in addition to TRPM7's critical roles in regulating cell migration and invasion, an upregulated miR-28-5p expression results in a significant decrease in glioma cell proliferation and migration.<sup>371,372</sup> Rap1b was reported to be a target of miR-28-5p and its expression level was downregulated. Therefore, it was demonstrated that TRPM7 targeting Rap1b signaling to suppress glioma cells' proliferation and invasion by upregulating miR-28-5p expression.<sup>371</sup>

It is widely accepted that Ca<sup>2+</sup> entry into the mitochondria is mediated by the activity of the mitochondrial calcium uniporter (MCU) complex, composed of the pore-forming subunit of the MCU channel together with several regulatory proteins. Abnormal changes in the expression of one or more members of the MCU complex have been associated with cancer-related phenotypes in HCC, breast cancer, colon cancer and pancreatic cancer.<sup>373</sup> Oncogenic miR-25 is highly expressed in prostate and colon cancer. miR-25 induces the downregulation of MCU with subsequent decreases in mitochondrial  $Ca^{2+}$  uptake and reductions in the apoptotic process of prostate and colon cancer. Importantly, miR-25-dependent reduction of mitochondrial  $Ca^{2+}$  can be rescued by miR-25 inhibitor.<sup>374</sup> In ovarian cancer, miR-195 contributes to regulating mitochondrial  $Ca^{2+}$  uptake in response to cytosolic  $Ca^{2+}$  concentration by repressing the mitochondrial calcium uptake 1 protein (MICU1).<sup>375</sup> Therefore, miRNAs play crucial roles in modulating intracellular  $Ca^{2+}$  signals in different cancer stages and types. Overall, the interplay between miRNAs and  $Ca^{2+}$  signaling in tumor microenvironments will offer novel therapeutic targets for the progress of targeted metastasis.

# 5.5 Molecular mechanism of the crosstalk between miRNAs and YAP

During metastasis, the disseminating cancer cells experience alterations in the microenvironment of cell-cell and cell-matrix stiffness.376 These different mechanical cues can be remembered by cells for long- or short-term periods, influencing the tumor cell phenotype in cancer progression.377 The Hippo pathway regulates cell proliferation, apoptosis, and stemness in response to a wide range of extracellular and intracellular signals.378 YAP/TAZ have been investigated in cancer and stem cells as mechanosensors in response to mechanical stimulation.379 Metastatic tumor cells retain their "mechanical memory" to acclimate to a new surface with a different stiffness during migration. The tumor cells containing YAP translocationdependent mechanical memory would lose the memory when YAP is depleted. Without YAP, cells migrate through the soft surface in the same way as through the stiff substrate. However, the roles of miRNAs in mechano-memory are poorly understood. Specifically, there exists a knowledge gap between miRNAs and Hippo-YAP/TAZ pathways in human malignancies.

Dysregulation of the Hippo-YAP signaling pathway underlies various solid tumors, and misregulation of miRNAs is a common feature in human cancers. Recent advances show that the Hippo-YAP signaling pathway affects the miRNA biogenesis by regulating the Microprocessor-interacting protein p72 and Dicer expression in a cell-density-dependent manner. At higher cell density, YAP translocates from the nucleus into the cytoplasm, thereby allowing p72 to bind to the Microprocessor in the nucleus and leading to efficient miRNA biogenesis.380 In contrast, at low cell densities, YAP stays in the nucleus and is activated, thereby sequestering p72 from the Microprocessor and disrupting the miRNA biogenesis.380 Cell-density induced translocation of nuclear YAP/TAZ represses the Dicer levels.<sup>381</sup> When nuclear YAP/TAZ are lost, levels of LIN28, a regulator of let7-a/b, is reduced. Lower LIN28 leads to let-7a and let-7b miRNAs accumulation, which down-regulates Dicer, resulting in decreased processing of pre-miRNA to mature miRNA (miR-23a, miR-22, miR-221, miR-24 and miR-21). Consistently, inhibition of let-7 rescues the miRNA biogenesis defects observed following YAP/TAZ knockdown (Fig. 5B and Table 3).<sup>381</sup>

The miR-130 family members, miR-130a and miR-130b, are located in chromosomes 11 and 22, respectively. Both miR-130a and miR-130b can mediate Hippo-YAP signaling in different cancers. Aberrant expression of miR-130a is observed in several types of cancer.<sup>382-385</sup> miR-130a is significantly down-regulated in HCC.<sup>384</sup> Conversely, miR-130a promotes YAP-induced liver tumorigenesis and liver enlargement in mice.<sup>386</sup> miR-130a can be induced as a direct target of the TEAD transcription complex, and the loss of endogenous YAP/TAZ substantially represses the pri- and mature miR-130a level. Also, miR-130a could effectively target VGLL4, an inhibitor of YAP. Therefore, aberrant YAP activation alone is enough to lead to liver tumorigenesis in a normal tissue microenvironment. The inhibition of miR-130a reversed liver size enlargement induced by Hippo pathway inactivation and blocked YAP-induced tumorigenesis.<sup>386</sup>

miR-130b, another member of the miR-130 family, induces the glioblastoma cancer stem cell phenotype through the regulation of the YAP/TAZ signaling pathway.<sup>387</sup> In the Hippo pathway, YAP/TAZ are phosphorylated and activated by kinase MST1/2 and LATS1/2 in mammals.<sup>388</sup> In addition, MST1/2 can bind to and phosphorylate the adaptor protein SAV1 and form MST1/2-SAV1 interaction to phosphorylate LATS1/2.<sup>389</sup> miR-130b is overexpressed in human glioblastoma and directly targets the MST1 and SAV1, resulting in the inactivation of the Hippo signaling pathway.<sup>387</sup> Hence, understanding the role of miR-130b in glioblastoma pathogenesis may shed light on novel therapeutic strategies.

miR-21 is overexpressed in most tumor types and acts as an oncogene by targeting many tumor suppressor genes related to proliferation, apoptosis, and invasion.<sup>390,391</sup> It has been demonstrated that miR-21 functions as a long-term mechanical memory keeper against different environmental mechanics, while YAP/TAZ primarily respond to acute changes of substrate mechanical cues in MSCs' migration.<sup>392</sup> In addition to MSCs, pancreatic cancer cells also commonly migrate through tissues of different stiffnesses during metastasis. Liver is the major metastatic site of pancreatic cancer. Metastatic niche in a softer environment presents a higher intrinsic resistance to gemcitabine monotherapy, a standard first-line treatment for patients with metastatic pancreatic cancer.<sup>393,394</sup> In pancreatic cancer, YAP nuclear translocation and miR-21 expression mediate the mechanical memory in response to altered environmental stiffness.<sup>393</sup> Meanwhile, environmental stiffness can influence the gemcitabine chemoresistance of soft-primed pancreatic cancer cells. These findings could shed light on how the regulation of miRNA expression affects tumor metastasis in patients, while miR-21 serve as a potential therapeutic target in metastatic tumor cells.

### 6. Conclusions and Outlook

In the past few decades, studies show that biophysical signals can regulate biochemical signaling in normal cells and cancer cells. Specifically, in response to biophysical inputs, (1) calciumrelated ion channels and transporters mediate calcium signaling and interact with cytoskeletal proteins to regulate cellular function; (2) the mechanotransduction carried out by the cytoskeleton and nucleus mediate the YAP shuttling to trigger changes in the transcription of downstream genes, affecting cellular functions; (3) calcium signals trigger changes in cytoskeleton force to interact with YAP signaling; (4) the expression level of miRNAs can either change in response to ECM mechanics or directly regulate the gene expression of ECM proteins; (5) miRNAs target the gene expression of calcium-related transporters/channels to regulate calcium dynamics; and (6) modulated by cell density, the interactions between nuclear YAP and Microprocessors regulate the maturation of diverse miRNAs, mediating cell behaviors.

Despite the significant advances in the understanding of mechanotransduction related to  $Ca^{2+}$ , YAP, and miRNAs, several important questions are unanswered and being actively studied: (1) What are the direct mechano-sensor and molecular mechanism responsible for biophysical-signal-induced calcium and YAP signaling? (2) How does miRNA mediate mechano-transduction? (3) miRNA degradation can be induced by some target RNAs through a pathway called target-directed miRNA degradation (TDMD). Does mechanotransduction influence the target RNA expression levels and induce miRNA degradation through TDMD?

Most importantly, further mechanobiological studies, leveraging the *in vivo* imaging,<sup>395-399</sup> CRISPR/Cas9 genome editing,<sup>400-402</sup> and data science,<sup>29,403-405</sup> could facilitate elucidating the roles and mechanisms of Ca<sup>2+</sup>/YAP/miRNA within mechanotranduction *in vivo* and empower the development of mechanomedicine for combinatorial cancer therapeutics.

### 7. Terminology of mechanics

Force (or load, *F*): physical interaction between two objects. It causes an object with mass (m) to accelerate (a), obeying the Newton's 2nd law F = ma.

Stress ( $\sigma$  or  $\tau$ ): the internal force (*F*) per unit area (*A*) in continuum medium. It includes normal stress ( $\sigma$ ) and shear stress ( $\tau$ ),  $\sigma$  or  $\tau = F/A$ . For normal stress (tension and compression), the direction of force is perpendicular to the surface of area. For shear stress, the direction of force is parallel to the surface of area.

Strain ( $\varepsilon$  or  $\gamma$ ): the change in length ( $\Delta l$ ) of an object with respect to the initial length (l). It includes normal strain ( $\varepsilon$ ) and shear strain ( $\gamma$ ),  $\varepsilon$  or  $\gamma = \Delta l/l$ . For normal strain, the directions of  $\Delta l$  (elongation is positive and shortening is negative) and l are in parallel. For shear strain, the directions of  $\Delta l$  and l are perpendicular to each other. No rigid body rotation is included.

Tension (*T*): outward force (*F*) in the direction normal to the surface per unit area (*A*) that causes a positive normal stress and strain, T = F/A.

Compression (or pressure, *P*): inward force (*F*) in the direction normal to the surface per unit area (*A*) that causes a negative normal stress and strain, P = F/A.

Stiffness (*k*): the elastic resistance offered by an object to deformation ( $\Delta l$ ) under an applied force (*F*),  $k = F/\Delta l$ .

Elastic modulus: a measure of the stiffness of an elastic object under an applied stress, defined as the slope of its stress-strain curve in the elastic deformation region. It includes Young's modulus (E) and Shear modulus (G),  $E = 2G(1 + \nu)$ , where  $\nu$  is the Poisson's ratio.

Young's modulus (E): the ratio of tensile/compressive stress ( $\sigma$ ) to normal strain ( $\varepsilon$ ) in the linear elastic region of a material,  $E = \sigma/\epsilon$ 

Shear modulus (G): the ratio of shear stress  $(\tau)$  to shear strain ( $\gamma$ ) of an elastic material,  $G = \tau / \gamma$ .

Cell traction (or traction force): the force per unit area exerted by the cell on substrates.

Cell contractility: the capability of a cell to contract the microenvironment. It can be evaluated by traction.

Adhesion: the molecular attraction force in the area of contact between dissimilar particles or objects that tend to cling to each other.

Porosity ( $\varphi$ ): the fraction of the void volume ( $V_V$ ) over the total volume ( $V_{\rm T}$ ) in a material,  $\varphi = V_{\rm V}/V_{\rm T}$ .

#### Nomenclature

ARP2/3	Actin-related protein 2/3
ATP	Adenosine-5'-triphosphate
ABC	ATP-binding cassette
AA	Arachidonic acid
AGO	Argonaute
ACI	Atherosclerotic cerebral infarction
AFM	Atomic force microscopy
BFGF	Basic fibroblast growth factor
BCL2	B-cell lymphoma 2
BRCA1	Breast and ovarian cancer susceptibility pro-
	tein 1
$Ca^{2+}$	Calcium ion
CW	Ca <sup>2+</sup> wave
CaMKII	Calcium/calmodulin-dependent protein kinase
	II
CICR	Calcium-induced calcium release
CalA	Calyculin A
CAF	Cancer-associated fibroblast
CLL	Chronic lymphocytic leukemia
CDS	Coding sequences
CRC	Colorectal cancer
Cx	Connexin
CXCR4	C-X-C Motif Chemokine Receptor 4
CDK4/6	Cyclin-dependent kinase 4/6
CytD	Cytochalasin D
[Ca <sup>2+</sup> ] <sub>cyt</sub>	Cytoplasmic Ca <sup>2+</sup> concentration
CAM	Cytoskeleton-actin-matrix
DRM	Detergent-resistant membrane
DGCR8	Digeorge critical region 8
ER	Endoplasmic reticulum
EGF	Epidermal growth factor
EPC	Epidermal stem/progenitor cell
EMT	Epithelial-mesenchymal transition

View Article Online
Soft Matter
Exportin
Extracellular matrix
Extracellular-signal-regulated kinase
Fibronectin
Fibronectin type III domain containing 3A
Filamentous-actin
Focal adhesion kinase
Globular-actin
G-protein-coupled receptor
Hepatocellular carcinoma
Homeobox A9
Human mesenchymal stem cell
Human umbilical vascular endothelial cell
Hydroxyl group
Inositol trisphosphate
Inositol trisphosphate receptor
Integrin-linked kinase
Intermediate filament
Intestinal epithelial cell
KRAS-induced actin-interacting protein
Large tumor suppressor 1/2
Linker of nucleoskeleton and cytoskeleton
Lin11, Isl-1 and Mec-3 kinase
Mammalian Ste20-like kinases 1/2
Matrix metallopeptidase 9
Meniscus fibrochondrocyte
Mesenchymal stem cell
Messenger RNA
MicroRNA
Mechanosensitive miRNA
Microtubule
Mitochondrial calcium uniporter

Mitochondrial calcium uptake 1 protein

Mitogen-activated protein kinase

Phosphatase and tensin homolog

Mouse embryonic fibroblast Mycobacterium tuberculosis

Normal endothelial cell

Phosphoinositide 3-kinase

Poly-A specific ribonuclease

Post-translational modification

Ras homolog family member A

Rho-associated protein kinase

RNA-induced silencing complex

Runt-related transcription factor

Receptor tyrosine kinase

Polydimethylsiloxane

Nuclear envelope

Phospholipase C

Polyacrylamide

Polymerase II

Precursor miRNA

Primary miRNA

Protein kinase B

Nucleotide

Phosphate

Fn

FNDC3A

F-actin

G-actin

GPCR

HCC

HOXA9

HMSC

HUVEC

OH

IP<sub>3</sub>

IP<sub>3</sub>R

ILK

IEC

KRAP

LINC

LIMK

MST1/2

MMP-9

MFC

MSC

MT

MCU

MEF

Mtb

NEC

NE

nt

Р

PTEN

PI3K

PLC

PAA

PARN

PDMS

Pol II

PTM

AKT

RhoA

ROCK

RTK

RISC

RUNX

pre-miRNA

pri-miRNA

MICU1 MAPK

mRNA

miRNA

mechanomiR

LATS1/2

IF

FAK

RyR	Ryanodine receptor
SUN	Sad1p-UNC-84
SERCA	Sarco/endoplasmic reticulum Ca <sup>2+</sup> ATPase
SPINK1	Serine peptidase inhibitor, Kazal type-1
SIRT1	Sirtuin 1
SFK	Src family kinase
SOCE	Store-operated Ca <sup>2+</sup> entry
STIM	Stromal interaction molecule
TDMD	Target-directed miRNA degradation
TG	Thapsigargin
TAZ	Transcriptional co-activator with PDZ-binding
	motif
TSS	Transcription start site
TRP	Transient receptor potential
TRPA	Transient receptor potential ankyrin
TRPC	Transient receptor potential canonical
TRPM	Transient receptor potential melastatin
TRPV	Transient receptor potential vanilloid
TPM	Tropomyosin
TEC	Tumor-derived endothelial cell
BTEC	TEC from human breast carcinomas
TNF	Tumor necrosis factor
TRAF6	TNF receptor associated factor 6
TRAIL	TNF-related apoptosis-inducing ligand
USP28	Ubiquitin-specific protease 28
UTR	Untranslated region
UCEC	Uterine corpus endometrial carcinoma
VEGF	Vascular endothelial growth factor
VGCC	Voltage-gated calcium channel
YAP	Yes-associated protein
TEAD	YAP-TEA domain
2-APB	2-Aminoethoxydiphenyl borate
m <sup>7</sup> G	7-methylguanosine

### Conflicts of interest

There are no conflicts to declare.

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