特约评述

DOI: 10.12211/2096-8280.2021-066

力信号在干细胞命运决定过程中的影响

宋成治[1]，孙阳[1]，曹毅[1,2]
（[1] 南京大学物理学院，江苏 南京 210093；[2] 南京大学固体微结构物理国家重点实验室，江苏 南京 210093）

摘要：干细胞因具有高效的自我更新能力和分化潜能而具有广泛的应用前景。随着对干细胞命运决定过程的研究不断深入，力信号作为在生物化学因子之外的又一重要影响因素渐渐走入研究者们的视野。对生长环境力学性质的影响效果和作用途径的研究对于深入理解干细胞分化选择的决定机制以及细胞内普遍存在的力学信号传导过程有着十分重要的意义。近年来，合成生物材料的发展极大拓宽了这一领域的研究手段，力学性能可控的合成材料使得条件设计和变量调节更加多元合理。其中，依托于基因重组等技术构建出的蛋白质水凝胶借助其良好的生物相容性和广阔的设计空间为深入挖掘力学性质影响背后的生物学机理提供了更多可能。本文主要介绍了力学信号对干细胞行为的影响效果和作用机理，同时还将介绍各种合成材料在干细胞分化研究领域内的重要应用。对力学信号和细胞行为更加定量化的调控和表征将会是这一领域深入发展的关键，利用合成生物学的方法构建更具针对性的研究工具意义重大。

关键词：干细胞；机械力信号；干性；分化

中图分类号：Q81　　**文献标志码：**A

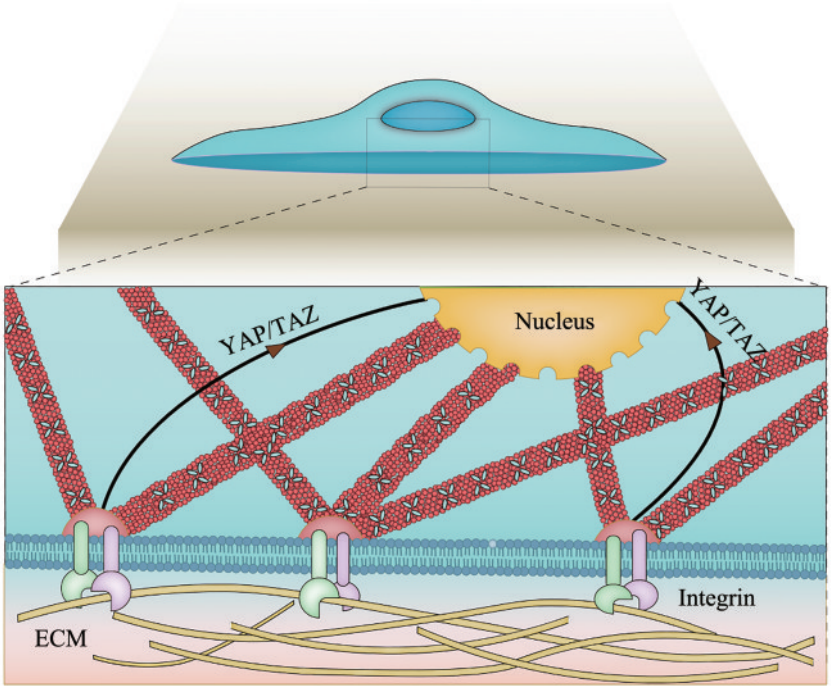
Effects of mechanical signals on stem cell fate determination

SONG Chengzhi[1]，SUN Yang[1]，CAO Yi[1,2]
（[1]*Department of physics*，*Nanjing University*，*Nanjing 210093*，*Jiangsu*，*China*；[2]*Nanjing Laboratory of Solid State Microstructure*，*Nanjing University*，*Nanjing 210093*，*Jiangsu*，*China*）

Abstract: Because of their outstanding self-renewing potential and pluripotency, stem cells are believed to have a broad range of applications in various fields such as tissue engineering, drug discovery, gene therapy, and cell therapy. While traditional studies in the field of stem cell fate determination mainly focused on bio-chemical factors, mechanical signals have come into sight of the science community as a crucial role in this area as well. A clearer understanding of the effect of mechanical forces and the mechanotransduction pathways in stem cells are surely helpful for their biomedical and clinic applications. Using synthetic hydrogels with well-defined rigidities as the substrates, the mechano-sensing mechanism and the lineage specification of stem cells have been extensively studied. Recent studies indicate that beyond the effect of substrate rigidity, other mechanical/physical properties, such as stress relaxation,

收稿日期：2021-06-09 修回日期：2021-10-30
基金项目：国家重点研发计划（2020YFA0908100）
引用本文：宋成治，孙阳，曹毅. 力信号在干细胞命运决定过程中的影响[J]. 合成生物学，2022，3(4)：781-794
Citation：SONG Chengzhi，SUN Yang，CAO Yi. Effects of mechanical signals on stem cell fate determination[J]. Synthetic Biology Journal，2022，3(4)：781-794

degradation, and porosities, are also critical to stem cell self-renewal and differentiation. However, it remains challenging to control these mechanical parameters of synthetic hydrogels precisely and independently. The advance in synthetic biology may provide novel synthetic protein hydrogels with controllable mechanical responses for the study of force sensing mechanisms and lineage specification of stem cells. In this review, we focus on the impact of mechanical signals on stem cell differentiation and the underlying molecular mechanism. The mechanical signals could be passed down to the nucleus either through a direct mechanical connection of ECM (extracellular matrix)-integrin-FA (focal adhesion)-cytoskeleton or through some signaling molecules. Meanwhile, we introduce some commonly used synthetic hydrogels systems that have been widely used as model systems for stem cell studies. We also highlight different mechanical responses of stem cells cultured in 2D and 3D. It is believed that precise characterization of the cellular behaviors and the mechanical signaling pathways are crucial and can be realized by constructing more specialized hydrogels using advanced synthetic biology tools.



Keywords: stem cell; mechanical signals; stemness; differentiation

干细胞的研究最早始于20世纪60年代，这一概念一经提出便很快得到人们的青睐。1981年Evans和Kaufman[1]成功实现了小鼠胚胎干细胞的分离与离体培养，进一步促进了这一领域研究热潮的形成。干细胞的备受瞩目源于其自身强大的自我更新和分化潜能以及基于此的广泛应用前景：作为细胞疗法的重要支柱，各种类型的干细胞为许多遗传病和退行性疾病的治疗提供了新的思路[2]。例如将造血干细胞移植用于骨髓系统重建，至今已使超过100万的病人从中获益[3]；干细胞也是组织工程的核心材料，目前通过组织工程人们已经可以构建出具有一定生理活性的组织器官如肺叶、心脏等[4-5]，而干细胞则为组织工程的实现提供了多样的材料选择。除胚胎干细胞外，一些专能干细胞在适当条件下也能够分化为多种不同的组织类型，具有极高的塑性[6]。另外在胚胎发育研究方面，干细胞的分化特性更是为人们理解发育提供了重要的参考模板[7]。但若想将干细胞

真正为人们所用，首先必须要理解其干性维持和分化调控的基本原理。在之前几十年的研究中，研究的重心多集中于各类生物化学因子本身的作用上[8-10]；一直到近些年，细胞外力学环境在干细胞命运决定过程中的作用才被逐渐解锁。随着合成生物材料的不断发展，力学性质可控的干细胞培养环境拓展了人们探究力信号作用的手段，通过设计凝胶或微柱阵列等体系的模量参数，可以实现对干细胞生存环境力学性质的定量调控，在不同的力信号下探究干细胞的行为，从而获得对干细胞分化调节机制更为全面的理解。

1 力信号对干细胞行为的影响

除化学生物因子外，机械力学信号也会对干细胞的干性维持和分化特性产生重要影响（图1）。首先，静态的力学信号可以调节干细胞的行为。细胞外基质的强度、黏弹性、塑形等力学性能的差异均可导致干细胞的不同响应。另外，动态的力学信号如外界的拉伸、压缩、扭转等也可影响干细胞行为，且其影响效果与力信号本身的频率、幅度、持续时长等多种因素有关。

1.1 基底模量大小对干细胞的影响

细胞感受到的环境力学信号首先便是基底的硬度。在人体中，不同组织的强度有很大的不同，如大脑的弹性模量只有约0.1～1 kPa（模量随位置不同会有一定变化）[11]，而骨骼的弹性模量可达到20 GPa[12]，生活在不同环境中的细胞从外界感受到的基底硬度信息差异很大，而这种环境的差异性自然表现为细胞的不同表型。实验表明，基底的硬度可以对组织细胞迁移、伸展、增殖等众多行为造成影响[13-17]，事实上，保持对于基底强度的动态感知是一般细胞维持存活的必要条件。如果基底过软或细胞对基底强度信息获得的途径中断，细胞便会很快走向凋亡[18]。对于干细胞而言，基底强度还可以直接影响细胞干性和分化方向：例如神经干细胞在模量处于100～500 Pa的基底上形成神经元，而在模量处于1000～10 000 Pa的基底上形成胶质细胞[19]；间充质干细胞在柔软环境中（微柱阵列弹性系数K=1.9 nN/μm）倾向于成脂分化而在较硬环境中（微柱阵列弹性系数K=1 556 nN/μm）则倾向于成骨分化[20]。

1.2 基底黏弹性与塑性对干细胞的影响

黏弹性用于刻画材料在发生形变时兼具弹性和黏性的特征。不同于一般的弹性物质，具备黏弹性的物质的应力响应与时间相关，在整个形变发生和恢复的过程中会同时带来能量的耗散。黏弹性的时间响应特征及其蕴含的塑性成分为细胞对基底的感知和重塑提供了更多机会，能够独立于基底强度对干细胞的行为产生影响[21-23]。基底塑性用来反应材料基底在外力影响下的塑性形变成分，它使培养环境可随着细胞的生长发生一定程度的形变，减小对细胞扩展的束缚[24-27]。实验研究发现，细胞外基质损耗模量增加所代表的塑性增强可以促进间充质干细胞的延展和分化[28-30]，促进有丝分裂和细胞周期的完成[31-32]。同时，在模量较小时塑性强的基底往往也更有利于干细胞的生长

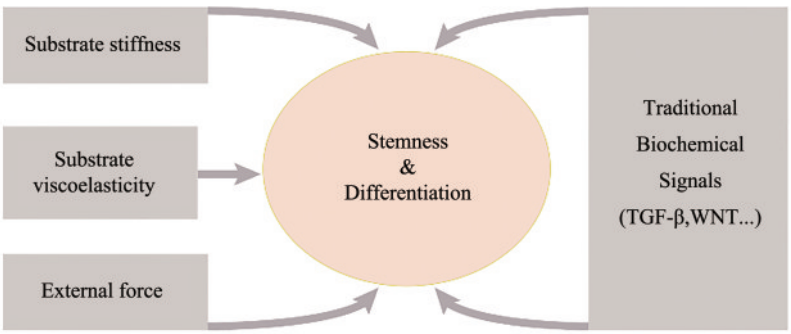


图1 力学信号对干细胞行为造成影响

Fig. 1 Mechanical signals affect stem cells behavior as traditional biochemical signals

和干性的维持[23]。事实上最早的关于基底硬度对组织再生领域干细胞分化调控的研究便是在黏弹性材料上完成的[33]，而硬度本身的调节作用也需要借助于对环境的重塑才可实现[34]。利用一种之前已有的抓手模型，可以大致重现细胞对基底硬度和黏弹性响应中的动力学过程[35-38]。许多水凝胶材料在软骨再生、声带重构、心肌梗塞病例模型的构建方面的应用便很可能依赖于其黏弹性和塑性特征[39-42]。

1.3 动态力学拉伸对干细胞的影响

在环境的静态力学特性之外，外界如拉伸或压缩这样的动态力学信号也会对细胞的行为产生重要影响。相比之下，动态力学信号的可变要素更多因而其作用效果也往往更为复杂，即使对于同种细胞，不同频率、振幅、间隔时长的力信号也可能会造成很不相同的结果[43]。动态力学信号的作用效果可以在一定程度上代替基底硬度的调节功能，对细胞的增殖、迁移、黏附、分化等带来显著影响[43-45]。值得说明的是，动态力学信号往往需要和生化因子相互结合才能发挥其对干细胞命运决定过程的影响。但二者结合作用的效果要比单独的力信号作用或生化因子作用更为明显，形成1+1＞2的调节特征[46]。例如动态的力学扭转和压缩可以显著促进间充质干细胞（MSC）的软骨发生[47]，如图2所示。相比于对照组，在幅度0.4 mm（10%～20%）、1 Hz的动态压缩作用下或者在转幅±25°、1 Hz的动态扭转作用下（两种作用时长均为每周连续5天，持续3周，每天1 h），MSC的成骨指标和成软骨指标表达量只出现一定程度的上升。但若将两种作用结合则可以看到两种指标在整个区域内均有显著增长（图2中最后一列图像整体颜色相比于第一列均更深）。另外，在与TGF-β3共同作用时，动态力学信号还可以进一

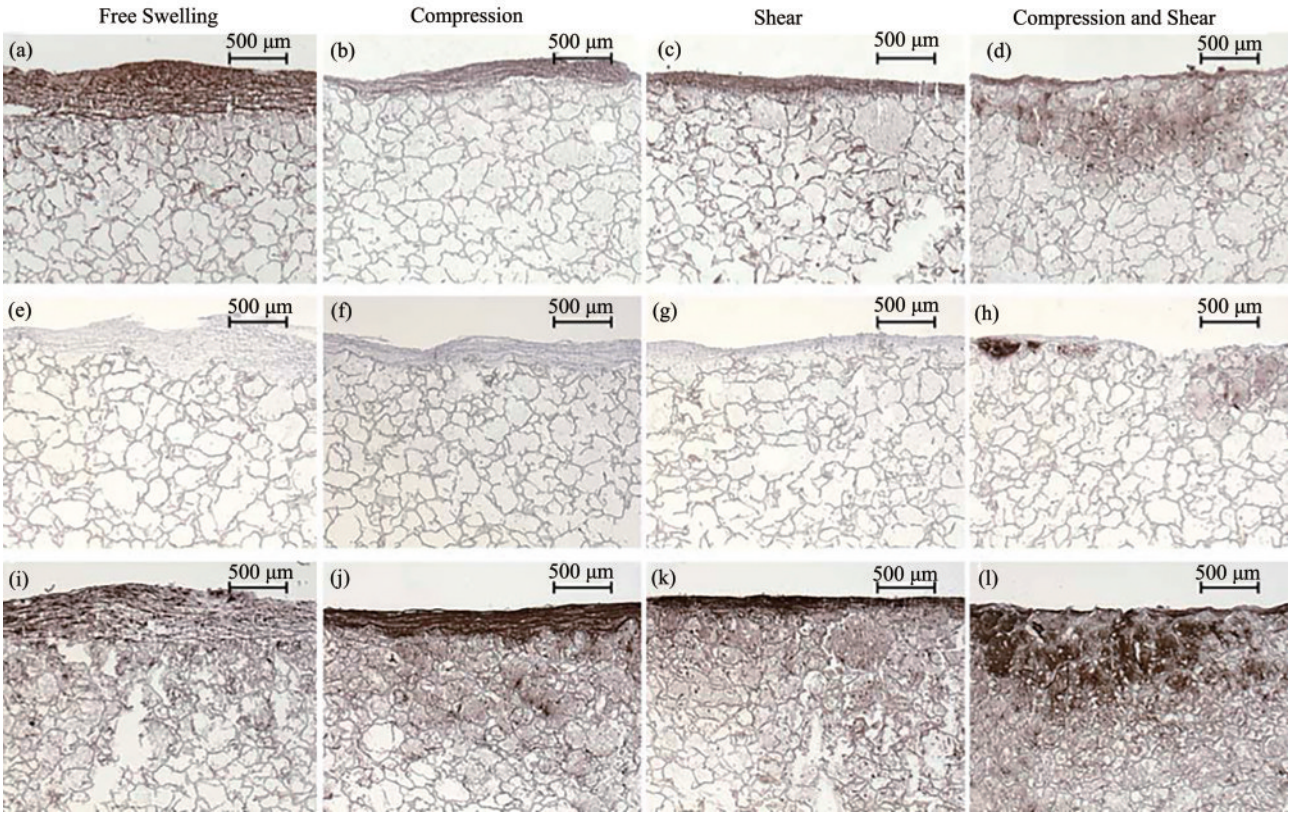


图2 不同加力方式处理下的MSC冰冻切片免疫荧光染色照片

[（a）～（d）为Agg染色状况，（e）～（h）为Col 2染色状况，（i）～（l）为Col 1染色状况。其中Agg与Col 2均为成软骨指标，Col 1为成骨指标[47]]

Fig. 2 Immunostaining for Col 1, Col 2, and Agg of MSC cryo-sections after different loading regimes

[Agg(a～d), Col 2(e～h), Col 1(i～l). Agg and Col 2 are chondrogenic markers while Col 1 are osteogenic markers.]

步提高人造软骨结构的力学性能[48]。扭转和压缩信号之所以能够促进软骨形成，很大一部分原因便在于这种外加的信号更好地模拟了体内软骨形成所需的真实生理环境，还原了结构形成的必要条件[49]。这种仿生的思想在生物材料设计的其他许多地方也常有体现。

2 力信号传导原理和相关通路分析

力信号传导主要需要经历3个步骤，首先需要细胞对周围的力学信号进行识别与感知，接着需要将细胞表面接受到的信号传导至细胞核，最后由细胞核通过基因表达调控来对外界的力学信号做出响应。在接受信号的第1步，常常需要对来自细胞外基质或细胞间的力学信号进行感知，通常借助于整合素、钙黏素等途径实现。而后续的信号传导则相对较为复杂，既有借助于信号分子的化学传导，又有借助于细胞骨架拉伸的机械传导。下面集中对上述两个环节中的主要原理进行简单的介绍。

2.1 力信号的感知与传导

2.1.1 细胞通过整合素和E-钙黏素来感知环境和周围细胞力信号

机体中细胞接收到的力信号主要有细胞外基质（ECM）和周围其他细胞两个来源[50]。对于ECM中的力学信号，其感知主要依赖于细胞膜上的跨膜蛋白整合素。整合素由α和β两个亚基构成，其胞外部分可同ECM中的配体（如RGD）结合感知细胞外基质的状态，而胞内部分则通过适配的蛋白与细胞骨架相连[18]（前面提到的分子抓手模型刻画的正是整合素和细胞骨架肌动蛋白间的偶联作用[51-52]）。当胞外结构域与特定蛋白结合时，其胞内部分也将发生构象变化并激活邻近黏着斑激酶（FAK）的作用，促进黏着斑的聚集。基底的强度不同，将会不同程度地激活FAK的活性[53]，进而表现为黏着斑强度和大小的差异[54-58]。事实上，细胞正是通过黏着斑的聚集或降解来达到与周围环境的动态平衡[59]。以细胞由悬浮状态变为黏附状态为例，细胞与基底接触后，整合素聚集成团簇，通过激活Src促进肌动蛋白的组装[60-61]。另外，在基底强度响应过程中，还会发生踝蛋白的酶解[62]、表皮生长因子受体（EGFR）的激活[63]、α-辅肌动蛋白的招募[64]、整合素αvβ3到α5β1的转变等过程[65]。细胞间依赖于钙黏蛋白的黏附和力信号感知过程与整合素介导的黏附类似，也需要经历黏着团簇形成、肌动蛋白交联等一系列过程来完成[66-73]。

2.1.2 细胞骨架动态组装和肌动蛋白收缩在力信号传导中的重要作用

在力信号的胞内传导过程中细胞骨架可以起到桥梁作用：由肌动蛋白等组成的细胞骨架外侧借助于和细胞膜上的黏着斑相连感受来自外部的力学信号刺激；内侧借助于同核骨架蛋白（LMNA）的锚连与细胞核相接，将细胞在表面感知到的物理信息转变为细胞核内部基因表达上的差异。当细胞通过拉伸肌动蛋白以感受周围环境的强度信息时，细胞骨架所产生的应力将带动内侧相连的LMNA一同运动，通过LMNA的拉伸和展开以及其表达水平来调节染色质的结构，促进转录因子的结合，进而对细胞的表型产生影响[74-75]。

2.1.3 细胞与细胞外基质的相互作用

细胞对于基底力学信号的感知往往需要借助于细胞边缘的标准感应模块的周期性形成与降解来完成[76]。细胞通过多轮的感知过程调整黏着斑的强度和大小以及自身细胞骨架的结构状况：较强的黏附会进一步促进更为活跃的伪足活动，加强与基质的联系[77]，并通过更多肌动蛋白拉力纤维与黏着斑的交联来促进整合素和细胞骨架间的连接[78]；而过软的基质则会使已经形成的黏附逐渐解聚，并可能通过死亡相关蛋白激酶1（DAPK1）相关通路的激活引发细胞的凋亡[79]。而在黏附强度变化的过程中细胞也会对ECM进行不同程度的重塑，ECM的强度变化将会进一步影响细胞的黏附状况和力感知过程，二者能够形成动态的相互影响。不过，短暂的力学感受过程（约几分钟的时间就可以完成一轮感知过程）也可以对细胞命运产生长时程的影响[80]，当力信号持续时间和强度达到一定阈值，干细胞能够通过YAP/TAZ的活动对之前的信号刺激产生记忆[81]，力信号传导如图3所示。

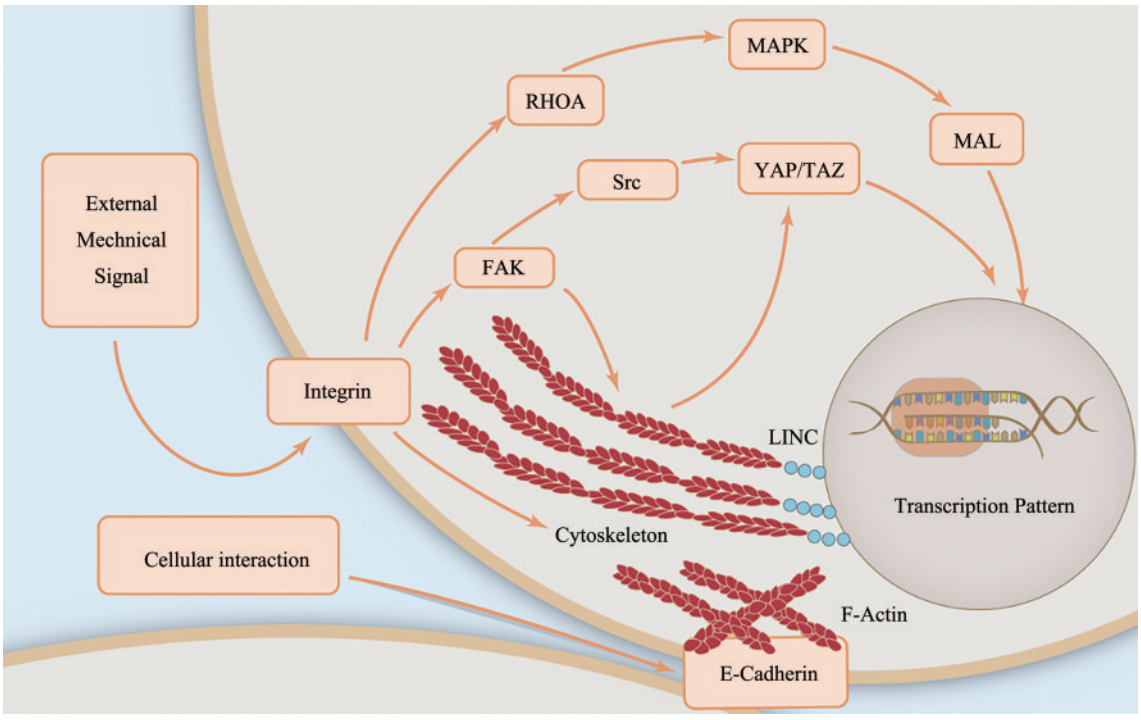


图3 力学信号传导过程

（细胞外力学信号可以通过细胞外基质-整合素-细胞骨架间的连接或借助YAP/TAZ等信号分子的传导进入细胞核中产生作用。而细胞间力学信号则通过E-Cadherin进行介导）

Fig. 3 Schematic diagrams for mechanical signal transduction

[External mechanical signal could be passed down to the nucleus either through a direct mechanical connection of ECM (extracellular matrix)-integrin-cytoskeleton or through some signaling molecules such as YAP/TAZ while cellular interaction is mediated through E-Cadherin (LINC: nucleoskeleton and cytoskeleton complex)]

2.2 YAP/TAZ通道在力学信号传导中的作用

细胞通过整合素等黏附蛋白感受到外界环境的力学特性后，会通过肌动球蛋白伸缩性的改变和细胞骨架的组织重构来与外界达到力学平衡[82]，与此同时力信号向细胞核方向的传播在很多情境中都需要借助于转录因子YAP（Yes-associated protein）与TAZ（transcriptional co-activator with PDZ-binding motif）的共同作用来实现[83-85]。YAP和TAZ均为Hippo信号通路中的末端效应物，作为Hippo通路的一部分，可以对组织生长的大小进行调控。除此之外，二者在上皮间充质转化、干细胞自我更新、肿瘤发育、环境力信号感知等过程中也起着重要作用[86-88]。另外，YAP和TAZ还可独立于Hippo通路发挥作用，例如转化生长因子TGF-β就可对这两种蛋白的行为直接进行调控[43, 89-90]。YAP/TAZ常被同时提及，主要是由于在大量试验条件和生物功能中二者在结构和对应基因特征上表达出的高度相似性。但二者在特定基因敲除的小鼠中可以分别导致不同的表型，暗示两者在信号通路中普遍相似的功能之外也存在各自独立的功能[91-92]，二者的差异性仍是目前学术界研究的一个重点。在较硬的基底中，黏着斑的增长和细胞骨架张力传导会促进YAP/TAZ的表达与核聚集现象；而在较软的基底中，YAP/TAZ的活动反而会受到抑制[93-94]。

YAP的激活需要黏着斑中黏着斑激酶（FAK）、Src的协助，其中Src既可以通过磷酸化YAP来实现直接的调节[95]，也可通过调节细胞骨架的结构来间接影响YAP/TAZ的功能，在不同背景下两种影响作用的主次关系尚待进一步探明。另外，整合素介导的YAP/TAZ激活可能也与β-PIX、小G蛋白Rac1、PAK等因素有关[96]。但对于肌动蛋白细胞骨架如何调控YAP/TAZ行为目前尚无定论，有研究认为血管生成素（AMOT）可能是二者联系的信使[97-98]，但这种观点目前尚缺少有

力的实验论据[99]。在黏附蛋白之外，其他的一些膜蛋白也可为YAP/TAZ通路传导力学信号，如神经干细胞中的钙离子通道Piezo1就可以激活YAP/TAZ的活动[100]。另外，细胞延展等因素也可在不借助于黏着斑的条件下影响YAP/TAZ通路的活动[101]，延展造成的细胞骨架收缩可通过与LINC蛋白复合物的相互作用来调节细胞核的行为。近年来有研究认为，YAP/TAZ的核聚集实际上正是通过细胞骨架调整核孔通透性而间接实现的，有实验已经观察到，当F-肌动蛋白与LINC复合物的锚连被切断时，YAP/TAZ的作用也被抑制[102]。

2.3 独立于YAP/TAZ之外的其他力信号传导通路

虽然许多的力信号传导途径均和YAP/TAZ相关，但部分通路也可独立于二者完成对外界力信号的感知。黏着斑、细胞骨架激活RHOA（Ras homolog family member A）和MAPK（mitogen-activated protein kinase）蛋白后，除了可以通过YAP/TAZ通路调节转录活动，还可借助于MAL蛋白来调节基因的表达。例如MAL下游的*JUNB*基因便可调节细胞的分化行为[103-104]。另外有实验表明，对乳腺癌病人的组织细胞进行体外三维培养的过程中，对基底硬度的感知无需通过YAP的核聚集来完成，提示我们在某些场景中力信号传导可以独立于YAP完成[105]。

2.4 Piezo蛋白与力信号感知与传导

Piezo蛋白家族为一类可将机械力学信号转化为电生理信号的跨膜蛋白[106]。其中Piezo1蛋白为三叶螺旋桨状的三聚体结构[107]，由于近年来其在力信号传导中的突出作用被人们所发现，Piezo蛋白开始受到越来越多的关注。Piezo可以对细胞膜上的张力进行独立响应，通过膜张力变化带来的门控通道开启改变膜两侧电位差以此完成力学信号的转换。例如在神经干细胞中，Piezo1钙离子通道便可对细胞膜上的张力做出响应，进而借助于YAP/TAZ通路来调节基因的表达状况。另外，Piezo蛋白的力学响应特征还使其在血红细胞体积调节、上皮细胞稳态维持、器官压力感受中起到重要作用[108]。

3 合成材料用于构建细胞生长环境

研究细胞外力学信号对干细胞命运决定过程的影响与机理，首先便要求能够对施加的机械信号进行定量调控。合成生物材料的发展为人造细胞外环境的构建提供了越来越多的选择和可能性，大大推动了研究的进程。

3.1 合成材料模拟不同硬度细胞外基质

目前对不同硬度基底的模拟主要采用的是水凝胶网络和微柱阵列两套方案。多种水凝胶网络体系均可模拟ECM环境并对干细胞进行培养，如传统的聚丙烯酰胺网络便可通过调节丙烯酰胺单体和交联点双丙烯酰胺的浓度来实现对基底模量的连续调控[109]，对于聚乙二醇等体系也有类似的模量控制特征[110-111]。然而过高的聚合物浓度常常可能会影响细胞的黏附特性，因而在模量调节的过程中常要结合实验，需要兼顾基底强度、细胞黏附性等多种要素的平衡。借助于微柱阵列也可实现对不同强度的细胞外基质的模拟[20]，通过改变微柱的高度与间距即可定量调节基底的模量[112]，同时通过跟踪微柱的位移还可以测量细胞施加在基底上力的大小[76, 113-114]。另外，微柱阵列的优点也体现在解除基底强度和黏性特征偶联这一方面，实现对强度或黏性的独立调控[20, 115]。除上述体系外，聚碳酸酯、静电纺支架等体系还可以较好地维持间充质干细胞干性，其特性或许可以为组织构建带来更多帮助[116-117]。

3.2 合成材料几何结构特征对干细胞行为的影响

不同形状的基底可以影响细胞的基因表达状况[118]、分化方向[119]等。有研究发现，将胚胎成纤维细胞培养于具有相等面积的三角形或圆形微结构（微结构面积与单细胞的伸展面积相当）以及具有相同形状不同长宽比的微结构的基板上时，细胞的基因表达状况出现明显差异[118]。利用具有不同几何特征的基底对巨噬细胞的形状进行约束可使其向不同方向分化（较大的长宽比可诱导巨噬细胞向M2型转变）[119]。基底形状对细胞行为的影响可能与细胞骨架伸缩性的改变[118]以及信号通

路中cAMP浓度、PIP2和PIP3的合成等因素有关[120-121]。另外，基底的几何结构特征可以对干细胞分化状况产生显著影响，例如通过构建不同取向的黏性纤维基底可以促进MSC不同程度的成软骨分化（当基底纤维延展角度与微流控平台流液方向垂直时最有利于向软骨方向的分化）[122]。通过改变羟基磷灰石基底盘片的平整程度可以使YAP/TAZ表达最优化进而促进MSC成骨方向的分化。对基底几何结构特征影响效果的探究首先要求我们能够制备不同几何结构的基底材料（如基底的不同形状、不同延展取向、不同平整度等），这便需要进一步发展更具针对性的生物材料合成手段。

3.3 塑性形变生物合成材料与干细胞的力学互动

天然细胞外基质中的纤维蛋白网络以及多糖网络被认为是黏弹性和塑性的主要贡献者[123]。传统的水凝胶体系可通过在聚丙烯酰胺中加入线性的丙烯酰胺链来模仿天然网络的这一特性[124]；而对于蛋白质水凝胶，可以直接在单体序列中引入基质金属蛋白酶的作用片段来达成网络可塑性的目标。此外，诸如p53二聚序列的插入还可在不影响网络化学键数目的前提下十分方便地为体系带来更多物理交联，从而实现网络耗散模量相较于弹性模量较为独立的调控[125]。目前许多在再生医学中较为成功的生物材料应用案例如胶原蛋白网络、透明质酸网络和其他的一些促进骨骼肌、肝脏、神经组织再生的培养网络普遍都是由物理交联构成的水凝胶体系，均具有较好的塑性和黏弹性特征[126-130]，提示我们塑性及与其相关的细胞与基质的力学互动在组织培养中的潜在重要性。不过，对于当前的许多材料，动态性的材料重塑仍是一大难题，通过有效整合更多的合成路径来更精确地控制凝胶网络的结构、组分和特定官能集团的位置并实现对凝胶网络的实时调控或许将会是未来进一步探索基底黏弹性和塑性影响效果的必经之路[131-133]。近年来，借助于Dronpa、CarHc、UVR8等蛋白在特定波长光照下解聚的特点制作出的多种光调控水凝胶网络已经在这一领域做出了成功的尝试[134-136]。

3.4 利用合成材料构建三维培养环境

细胞培养的维数会对干细胞行为产生很大的影响，一些在二维培养条件下出现的现象，在三维培养时可能迥乎不同：例如三维环境中，乳腺癌细胞的力信号传导将不再依赖于二维条件下的YAP通路[105]；二维培养中不会出现的组织形态特征和分化表型在三维培养时能够产生[137]；在基底硬度的影响方面，二维培养和三维培养的细胞表现也会有所不同[138]。三维培养环境因其更接近于细胞生长的自然状态，对于细胞的形态学变化、干性维持等均有重要影响[139]，然而天然的细胞外基质如基底膜或Ⅰ型胶原蛋白由于难以调控其不同组分之间的关联，不便于研究特定力学信号对干细胞行为的影响，这时合成生物学材料在变量控制方面的优越性便体现出来：例如在基于SpyCatcher/tag化学的水凝胶网络中，通过基因重组技术在单体序列中插入更多的成键片段（如在两段ELP序列中间额外增加可用于成键的一段Spytag序列），便可实现对体系成键密度的调控，构建模量不同的细胞三维培养环境[125]（图4）。

4 前景展望

随着研究的不断深入，力信号对干细胞行为所起的调节作用正逐渐为人们所认知。合成生物材料的发展为这一领域的研究提供了更多的方法与手段。借助于水凝胶系统和微柱阵列，可以对细胞培养环境的单一力学特征进行半定量调节，在有效控制变量的基础上探明基底强度、黏弹性、塑形等特定力学性能对干细胞行为的具体影响。其中，蛋白质水凝胶由于其高度的生物相容性和巨大的设计空间正逐渐受到越来越多的关注，通过合理地对单体序列进行设计，可以方便地实现变量调控、动态结构转变等多种功能，为开展定量化的实验研究提供有利条件。另外，通过新型合成生物材料，还可以在三维环境下对干细胞进行培养和研究，探明在更接近于体内真实情景下力信号对干细胞干性维持和分化特性产生的调节作用。但同时也应当注意到，虽然在现象和机理上的认识不断深化，但仍有大量的未知内容有待

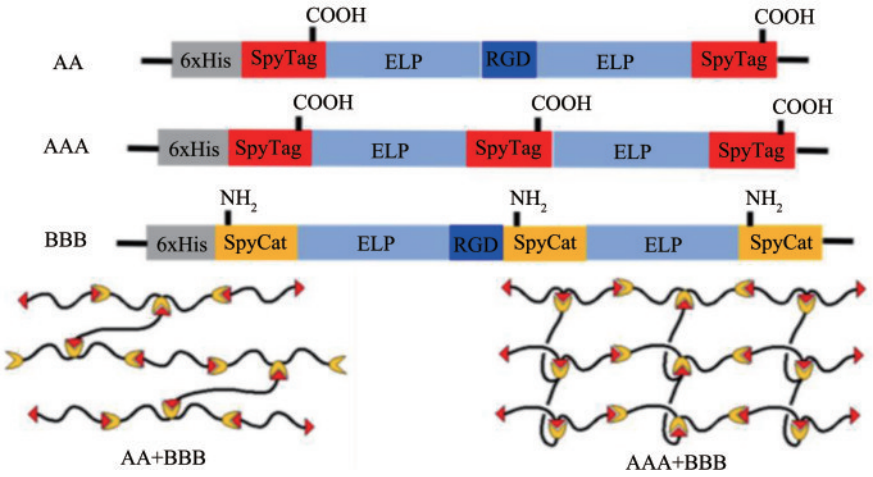


图4 利用Spycatcher/tag化学构建成键密度不同的水凝胶网络[125]

Fig. 4 Building hydrogel network with different bonding density using Spycatcher/tag chemistry[125]

进一步研究。一方面，需要从微观入手，研究力信号传导每一步的具体实现机理，确立某一节点调节背后的定量关系；另一方面，还需要从宏观上更准确地把握力信号影响所依赖的完整图景，从不同甚至截然相反的实验结果中提取出核心信息和调节途径，并在复杂交错的调控网络中理清各个节点间的联系。对于前者仍需要更为定量的实验方案：首先要能对细胞外环境的各类力学性能进行独立而精确的调控，另外还要能对细胞及其外环境的状态进行实时的监控与表征。不断发展的合成材料在细胞作用力的测量上就为实验设计提供了不少选择，从20世纪的硅类基底的褶皱状况辨识[140]，到如今的弹性凝胶系统中镶嵌的荧光小珠的移动分析[17, 141]、微柱阵列的位移表征[76, 112-114]，合成材料的使用可在已有仪器的基础上使力信号的施加与读取更加准确便捷。同时，原子力显微镜、结构光显微镜、荧光共振能量转移等仪器和新技术在力信号测量、核心分子活动特征分析等方面的合理使用也可以为更加精准定量的实验研究提供方便[142-145]。此外，分子动力学模拟等计算机方法的引入还可以帮助我们对核心分子在信号传导过程中的动力学行为进行直观刻画[146]。对于后者，实验数据的分析和要素提取则显得尤为重要，传统系统生物学的计算方法与机器学习结合，或许可以为完整调控网络的建立带来更多启示。认清机械力学信号对干细胞命运决定过程的影响意义非凡，无论是深入理解胚胎发育，还是推进未来组织工程发展和相关的靶向药物设计，其机制的探明都是无法绕开的核心话题，蕴藏着巨大的科研价值与产业化潜力，这一领域的研究定会因此而不断深入下去。

参 考 文 献

[1] EVANS M J, KAUFMAN M H. Establishment in culture of pluripotential cells from mouse embryos[J]. Nature, 1981, 292 (5819): 154-156.

[2] DE LUCA M, AIUTI A, COSSU G, et al. Advances in stem cell research and therapeutic development[J]. Nature Cell Biology, 2019, 21(7): 801-811.

[3] CHABANNON C, KUBALL J, BONDANZA A, et al. Hematopoietic stem cell transplantation in its 60 s: a platform for cellular therapies[J]. Science Translational Medicine, 2018, 10(436): eaap9630.

[4] REN X, MOSER P T, GILPIN S E, et al. Engineering pulmonary vasculature in decellularized rat and human lungs[J]. Nature Biotechnology, 2015, 33(10): 1097-1102.

[5] OTT H C, MATTHIESEN T S, GOH S K, et al. Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart[J]. Nature Medicine, 2008, 14(2): 213-221.

[6] JIANG Y H, JAHAGIRDAR B N, REINHARDT R L, et al. Pluripotency of mesenchymal stem cells derived from adult marrow[J]. Nature, 2002, 418(6893): 41-49.

[7] FU J P, WARMFLASH A, LUTOLF M P. Stem-cell-based embryo models for fundamental research and translation[J]. Nature Materials, 2021, 20(2): 132-144.

[8] DISCHER D E, MOONEY D J, ZANDSTRA P W. Growth factors, matrices, and forces combine and control stem cells[J]. Science, 2009, 324(5935): 1673-1677.

[9] CAMPBELL P A, PEREZ-IRATXETA C, ANDRADE-NAVARRO M A, et al. Oct4 targets regulatory nodes to modulate

stem cell function[J]. PLoS One, 2007, 2(6): e553.
[10] GRECO S J, LIU K, RAMESHWAR P. Functional similarities among genes regulated by OCT4 in human mesenchymal and embryonic stem cells[J]. Stem Cells, 2007, 25(12): 3143-3154.
[11] ENGLER A J, SEN S, SWEENEY H L, et al. Matrix elasticity directs stem cell lineage specification[J]. Cell, 2006, 126(4): 677-689.
[12] RHO J Y, ASHMAN R B, TURNER C H. Young's modulus of trabecular and cortical bone material: ultrasonic and microtensile measurements[J]. Journal of Biomechanics, 1993, 26(2): 111-119.
[13] BANGASSER B L, SHAMSAN G A, CHAN C E, et al. Shifting the optimal stiffness for cell migration[J]. Nature Communications, 2017, 8: 15313.
[14] VOGEL V, SHEETZ M. Local force and geometry sensing regulate cell functions[J]. Nature Reviews Molecular Cell Biology, 2006, 7(4): 265-275.
[15] DISCHER D E, JANMEY P, WANG Y L. Tissue cells feel and respond to the stiffness of their substrate[J]. Science, 2005, 310(5751): 1139-1143.
[16] ENGLER A, BACAKOVA L, NEWMAN C, et al. Substrate compliance versus ligand density in cell on gel responses[J]. Biophysical Journal, 2004, 86(1): 617-628.
[17] PELHAM R J JR, WANG Y L. Cell locomotion and focal adhesions are regulated by substrate flexibility[J]. Proceedings of the National Academy of Sciences of the United States of America, 1997, 94(25): 13661-13665.
[18] WOLFENSON H, YANG B, SHEETZ M P. Steps in mechanotransduction pathways that control cell morphology[J]. Annual Review of Physiology, 2019, 81: 585-605.
[19] SAHA K, KEUNG A J, IRWIN E F, et al. Substrate modulus directs neural stem cell behavior[J]. Biophysical Journal, 2008, 95(9): 4426-4438.
[20] FU J P, WANG Y K, YANG M T, et al. Mechanical regulation of cell function with geometrically modulated elastomeric substrates[J]. Nature Methods, 2010, 7(9): 733-736.
[21] CHAUDHURI O, COOPER-WHITE J, JANMEY P A, et al. Effects of extracellular matrix viscoelasticity on cellular behaviour[J]. Nature, 2020, 584(7822): 535-546.
[22] LEE H P, GU L, MOONEY D J, et al. Mechanical confinement regulates cartilage matrix formation by chondrocytes[J]. Nature Materials, 2017, 16(12): 1243-1251.
[23] MADL C M, LESAVAGE B L, DEWI R E, et al. Maintenance of neural progenitor cell stemness in 3D hydrogels requires matrix remodelling[J]. Nature Materials, 2017, 16(12): 1233-1242.
[24] CAIAZZO M, OKAWA Y, RANGA A, et al. Defined three-dimensional microenvironments boost induction of pluripotency[J]. Nature Materials, 2016, 15(3): 344-352.
[25] PAUL C D, MISTRIOTIS P, KONSTANTOPOULOS K. Cancer cell motility: lessons from migration in confined spaces[J]. Nature Reviews Cancer, 2017, 17(2): 131-140.
[26] SCHULTZ K M, KYBURZ K A, ANSETH K S. Measuring dynamic cell-material interactions and remodeling during 3D human mesenchymal stem cell migration in hydrogels[J]. Proceedings of the National Academy of Sciences of the United States of America, 2015, 112(29): E3757-E3764.
[27] WISDOM K M, ADEBOWALE K, CHANG J L, et al. Matrix mechanical plasticity regulates cancer cell migration through confining microenvironments[J]. Nature Communications, 2018, 9: 4144.
[28] CAMERON A R, FRITH J E, COOPER-WHITE J J. The influence of substrate creep on mesenchymal stem cell behaviour and phenotype[J]. Biomaterials, 2011, 32(26): 5979-5993.
[29] CHAUDHURI O, GU L, DARNELL M, et al. Substrate stress relaxation regulates cell spreading[J]. Nature Communications, 2015, 6: 6364.
[30] CHAUDHURI O, GU L, KLUMPERS D, et al. Hydrogels with tunable stress relaxation regulate stem cell fate and activity[J]. Nature Materials, 2016, 15(3): 326-334.
[31] NAM S, GUPTA V K, LEE H P, et al. Cell cycle progression in confining microenvironments is regulated by a growth-responsive $TRPV_4$-PI3K/Akt-p27 Kip1 signaling axis[J]. Science Advances, 2019, 5(8): eaaw6171.
[32] NAM S, CHAUDHURI O. Mitotic cells generate protrusive extracellular forces to divide in three-dimensional microenvironments[J]. Nature Physics, 2018, 14(6): 621-628.
[33] HUEBSCH N, LIPPENS E, LEE K, et al. Matrix elasticity of void-forming hydrogels controls transplanted-stem-cell-mediated bone formation[J]. Nature Materials, 2015, 14(12): 1269-1277.
[34] HUEBSCH N, ARANY P R, MAO A S, et al. Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate[J]. Nature Materials, 2010, 9(6): 518-526.
[35] BANGASSER B L, ROSENFELD S S, ODDE D J. Determinants of maximal force transmission in a motor-clutch model of cell traction in a compliant microenvironment[J]. Biophysical Journal, 2013, 105(3): 581-592.
[36] ELOSEGUI-ARTOLA A, ORIA R, CHEN Y F, et al. Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity[J]. Nature Cell Biology, 2016, 18(5): 540-548.
[37] GONG Z, SZCZESNY S E, CALIARI S R, et al. Matching material and cellular timescales maximizes cell spreading on viscoelastic substrates[J]. Proceedings of the National Academy of Sciences of the United States of America, 2018, 115(12): E2686-E2695.
[38] UROZ M, GARCIA-PUIG A, TEKELI I, et al. Traction forces at the cytokinetic ring regulate cell division and polyploidy in the migrating zebrafish epicardium[J]. Nature Materials, 2019, 18(9): 1015-1023.
[39] ATALA A, KIM W, PAIGE K T, et al. Endoscopic treatment of vesicoureteral reflux with a chondrocyte-alginate suspension[J].

The Journal of Urology, 1994, 152(2): 641-643.

[40] CHHETRI D K, MENDELSOHN A H. Hyaluronic acid for the treatment of vocal fold scars[J]. Current Opinion in Otolaryngology & Head and Neck Surgery, 2010, 18(6): 498-502.

[41] LIN X, LIU Y, BAI A B, et al. A viscoelastic adhesive epicardial patch for treating myocardial infarction[J]. Nature Biomedical Engineering, 2019, 3(8): 632-643.

[42] RUVINOV E, COHEN S. Alginate biomaterial for the treatment of myocardial infarction: progress, translational strategies, and clinical outlook: from ocean algae to patient bedside[J]. Advanced Drug Delivery Reviews, 2016, 96: 54-76.

[43] CUI Y D, HAMEED F M, YANG B, et al. Cyclic stretching of soft substrates induces spreading and growth[J]. Nature Communications, 2015, 6: 6333.

[44] SAHA S, JI L, DE PABLO J J, et al. Inhibition of human embryonic stem cell differentiation by mechanical strain[J]. Journal of Cellular Physiology, 2006, 206(1): 126-137.

[45] LI C W, LAU Y T, LAM K L, et al. Mechanically induced formation and maturation of 3D-matrix adhesions (3DMAs) in human mesenchymal stem cells[J]. Biomaterials, 2020, 258: 120292.

[46] MAUCK R L, NICOLL S B, SEYHAN S L, et al. Synergistic action of growth factors and dynamic loading for articular cartilage tissue engineering[J]. Tissue Engineering, 2003, 9(4): 597-611.

[47] SCHÄTTI O, GRAD S, GOLDHAHN J, et al. A combination of shear and dynamic compression leads to mechanically induced chondrogenesis of human mesenchymal stem cells[J]. European Cells & Materials, 2011, 22: 214-225.

[48] HUANG A H, FARRELL M J, KIM M, et al. Long-term dynamic loading improves the mechanical properties of chondrogenic mesenchymal stem cell-laden hydrogel[J]. European Cells & Materials, 2010, 19: 72-85.

[49] BAKER B M, SHAH R P, HUANG A H, et al. Dynamic tensile loading improves the functional properties of mesenchymal stem cell-laden nanofiber-based fibrocartilage[J]. Tissue Engineering Part A, 2011, 17(9/10): 1445-1455.

[50] ISKRATSCH T, WOLFENSON H, SHEETZ M P. Appreciating force and shape-the rise of mechanotransduction in cell biology[J]. Nature Reviews Molecular Cell Biology, 2014, 15(12): 825-833.

[51] SWAMINATHAN V, WATERMAN C M. The molecular clutch model for mechanotransduction evolves[J]. Nature Cell Biology, 2016, 18(5): 459-461.

[52] MITCHISON T, KIRSCHNER M. Cytoskeletal dynamics and nerve growth[J]. Neuron, 1988, 1(9): 761-772.

[53] VINING K H, MOONEY D J. Mechanical forces direct stem cell behaviour in development and regeneration[J]. Nature Reviews Molecular Cell Biology, 2017, 18(12): 728-742.

[54] HAMADI A, BOUALI M, DONTENWILL M, et al. Regulation of focal adhesion dynamics and disassembly by phosphorylation of FAK at tyrosine 397[J]. Journal of Cell Science, 2005, 118(Pt 19): 4415-4425.

[55] PRAGER-KHOUTORSKY M, LICHTENSTEIN A, KRISHNAN R, et al. Fibroblast polarization is a matrix-rigidity-dependent process controlled by focal adhesion mechanosensing[J]. Nature Cell Biology, 2011, 13(12): 1457-1465.

[56] TRICHET L, LE DIGABEL J, HAWKINS R J, et al. Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness[J]. Proceedings of the National Academy of Sciences of the United States of America, 2012, 109 (18): 6933-6938.

[57] VICENTE-MANZANARES M, ZARENO J, WHITMORE L, et al. Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells (vol 176, pg 573, 2007)[J]. Journal of Cell Biology, 2007, 176(7): 1073.

[58] WOLFENSON H, BERSHADSKY A, HENIS Y I, et al. Actomyosin-generated tension controls the molecular kinetics of focal adhesions[J]. Journal of Cell Science, 2011, 124(Pt 9): 1425-1432.

[59] WENG S N, SHAO Y, CHEN W Q, et al. Mechanosensitive subcellular rheostasis drives emergent single-cell mechanical homeostasis[J]. Nature Materials, 2016, 15(9): 961-967.

[60] CHOI C K, VICENTE-MANZANARES M, ZARENO J, et al. Actin and alpha-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner[J]. Nature Cell Biology, 2008, 10(9): 1039-1050.

[61] YU C H, LAW J B K, SURYANA M, et al. Early integrin binding to Arg-Gly-Asp peptide activates actin polymerization and contractile movement that stimulates outward translocation[J]. Proceedings of the National Academy of Sciences of the United States of America, 2011, 108(51): 20585-20590.

[62] SAXENA M, CHANGEDE R, HONE J, et al. Force-induced calpain cleavage of talin is critical for growth, adhesion development, and rigidity sensing[J]. Nano Letters, 2017, 17(12): 7242-7251.

[63] SAXENA M, LIU S M, YANG B, et al. EGFR and HER2 activate rigidity sensing only on rigid matrices[J]. Nature Materials, 2017, 16(7): 775-781.

[64] ROCA-CUSACHS P, DEL RIO A, PUKLIN-FAUCHER E, et al. Integrin-dependent force transmission to the extracellular matrix by α-actinin triggers adhesion maturation[J]. Proceedings of the National Academy of Sciences of the United States of America, 2013, 110(15): E1361-E1370.

[65] ZAMIR E, KATZ M, POSEN Y, et al. Dynamics and segregation of cell-matrix adhesions in cultured fibroblasts[J]. Nature Cell Biology, 2000, 2(4): 191-196.

[66] ACHARYA B R, WU S K, LIEU Z Z, et al. Mammalian diaphanous 1 mediates a pathway for E-cadherin to stabilize epithelial barriers through junctional contractility[J]. Cell Reports, 2017, 18(12): 2854-2867.

[67] BUCKLEY C D, TAN J Y, ANDERSON K L, et al. Cell adhe-

sion. The minimal cadherin-catenin complex binds to actin filaments under force[J]. Science, 2014, 346(6209): 1254211.

[68] COLLINS C, DENISIN A K, PRUITT B L, et al. Changes in E-cadherin rigidity sensing regulate cell adhesion[J]. Proceedings of the National Academy of Sciences of the United States of America, 2017, 114(29): E5835-E5844.

[69] NEJSUM L N, NELSON W J. Epithelial cell surface polarity: The early steps[J]. Frontiers in Bioscience (Landmark Edition), 2009, 14(3): 1088-1098.

[70] STRALE P O, DUCHESNE L, PEYRET G, et al. The formation of ordered nanoclusters controls cadherin anchoring to actin and cell-cell contact fluidity (vol 210, pg 333, 2015) [J]. Journal of Cell Biology, 2015, 210(6): 1033.

[71] WU S K, BUDNAR S, YAP A S, et al. Pulsatile contractility of actomyosin networks organizes the cellular cortex at lateral cadherin junctions[J]. European Journal of Cell Biology, 2014, 93(10/11/12): 396-404.

[72] WU Y, KANCHANAWONG P, ZAIDEL-BAR R. Actin-delimited adhesion-independent clustering of E-cadherin forms the nanoscale building blocks of adherens junctions[J]. Developmental Cell, 2015, 32(2): 139-154.

[73] REDDY P, LIU L, REN C, et al. Formation of E-cadherin-mediated cell-cell adhesion activates AKT and mitogen activated protein kinase *via* phosphatidylinositol 3 kinase and ligand-independent activation of epidermal growth factor receptor in ovarian cancer cells[J]. Molecular Endocrinology, 2005, 19(10): 2564-2578.

[74] SWIFT J, IVANOVSKA I L, BUXBOIM A, et al. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation[J]. Science, 2013, 341(6149): 1240104.

[75] TAJIK A, ZHANG Y J, WEI F X, et al. Transcription upregulation *via* force-induced direct stretching of chromatin[J]. Nature Materials, 2016, 15(12): 1287-1296.

[76] WOLFENSON H, MEACCI G, LIU S M, et al. Tropomyosin controls sarcomere-like contractions for rigidity sensing and suppressing growth on soft matrices[J]. Nature Cell Biology, 2016, 18(1): 33-42.

[77] GIANNONE G, DUBIN-THALER B J, ROSSIER O, et al. Lamellipodial actin mechanically links myosin activity with adhesion-site formation[J]. Cell, 2007, 128(3): 561-575.

[78] NAUMANEN P, LAPPALAINEN P, HOTULAINEN P. Mechanisms of actin stress fibre assembly[J]. Journal of Microscopy, 2008, 231(3): 446-454.

[79] IVANOVSKA J, MAHADEVAN V, SCHNEIDER-STOCK R. DAPK and cytoskeleton-associated functions[J]. Apoptosis, 2014, 19(2): 329-338.

[80] BALESTRINI J L, CHAUDHRY S, SARRAZY V, et al. The mechanical memory of lung myofibroblasts[J]. Integrative Biology, 2012, 4(4): 410-421.

[81] YANG C, TIBBITT M W, BASTA L, et al. Mechanical memory and dosing influence stem cell fate[J]. Nature Materials, 2014, 13(6): 645-652.

[82] VOGEL V, SHEETZ M. Local force and geometry sensing regulate cell functions[J]. Nature Reviews Molecular Cell Biology, 2006, 7(4): 265-275.

[83] GASPAR P, TAPON N. Sensing the local environment: actin architecture and Hippo signalling[J]. Current Opinion in Cell Biology, 2014, 31: 74-83.

[84] PANCIERA T, AZZOLIN L, CORDENONSI M, et al. Mechanobiology of YAP and TAZ in physiology and disease[J]. Nature Reviews Molecular Cell Biology, 2017, 18(12): 758-770.

[85] TOTARO A, PANCIERA T, PICCOLO S. YAP/TAZ upstream signals and downstream responses[J]. Nature Cell Biology, 2018, 20(8): 888-899.

[86] MILLER C J, DAVIDSON L A. The interplay between cell signalling and mechanics in developmental processes[J]. Nature Reviews Genetics, 2013, 14(10): 733-744.

[87] HENG B C, ZHANG X H, AUBEL D, et al. An overview of signaling pathways regulating YAP/TAZ activity[J]. Cellular and Molecular Life Sciences: CMLS, 2021, 78(2): 497-512.

[88] POCATERRA A, ROMANI P, DUPONT S. YAP/TAZ functions and their regulation at a glance[J]. Journal of Cell Science, 2020, 133(2): jcs230425.

[89] MEIER E, LAM M T. Role of mechanical stimulation in stem cell differentiation[J]. Biotechnology and Bioengineering, 2016, 3(3): 1069.

[90] SAHA S, JI L, DE PABLO J J, et al. TGFbeta/Activin/Nodal pathway in inhibition of human embryonic stem cell differentiation by mechanical strain[J]. Biophysical Journal, 2008, 94(10): 4123-4133.

[91] MAKITA R, UCHIJIMA Y, NISHIYAMA K, et al. Multiple renal cysts, urinary concentration defects, and pulmonary emphysematous changes in mice lacking TAZ[J]. American Journal of Physiology Renal Physiology, 2008, 294(3): F542-F553.

[92] MORIN-KENSICKI E M, BOONE B N, HOWELL M, et al. Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65[J]. Molecular and Cellular Biology, 2006, 26(1): 77-87.

[93] ARAGONA M, PANCIERA T, MANFRIN A, et al. A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors[J]. Cell, 2013, 154(5): 1047-1059.

[94] DUPONT S, MORSUT L, ARAGONA M, et al. Role of YAP/TAZ in mechanotransduction[J]. Nature, 2011, 474(7350): 179-183.

[95] ROSENBLUH J, NIJHAWAN D, COX A G, et al. β-Catenin-driven cancers require a YAP1 transcriptional complex for survival and tumorigenesis[J]. Cell, 2012, 151(7): 1457-1473.

[96] SERO J E, BAKAL C. Multiparametric analysis of cell shape demonstrates that β-PIX directly couples YAP activation to extracellular matrix adhesion[J]. Cell Systems, 2017, 4(1): 84-96.e6.

[97] ZHAO B, LI L, LU Q, et al. Angiomotin is a novel Hippo pathway component that inhibits YAP oncoprotein[J]. Genes & Development, 2011, 25(1): 51-63.

[98] MANA-CAPELLI S, PARAMASIVAM M, DUTTA S, et al. Angiomotins link F-actin architecture to Hippo pathway signaling[J]. Molecular Biology of the Cell, 2014, 25(10): 1676-1685.

[99] KIRBY T J, LAMMERDING J. Emerging views of the nucleus as a cellular mechanosensor[J]. Nature Cell Biology, 2018, 20(4): 373-381.

[100] PATHAK M M, NOURSE J L, TRAN T, et al. Stretch-activated ion channel Piezo1 directs lineage choice in human neural stem cells[J]. Proceedings of the National Academy of Sciences of the United States of America, 2014, 111(45): 16148-16153.

[101] ZHAO B, LI L, WANG L, et al. Cell detachment activates the Hippo pathway *via* cytoskeleton reorganization to induce anoikis[J]. Genes & Development, 2012, 26(1): 54-68.

[102] ELOSEGUI-ARTOLA A, ANDREU I, BEEDLE A E M, et al. Force triggers YAP nuclear entry by regulating transport across nuclear pores[J]. Cell, 2017, 171(6): 1397-1410.e14.

[103] CONNELLY J T, GAUTROT J E, TRAPPMANN B, et al. Actin and serum response factor transduce physical cues from the microenvironment to regulate epidermal stem cell fate decisions[J]. Nature Cell Biology, 2010, 12(7): 711-718.

[104] VARTIAINEN M K, GUETTLER S, LARIJANI B, et al. Nuclear actin regulates dynamic subcellular localization and activity of the SRF cofactor MAL[J]. Science, 2007, 316(5832): 1749-1752.

[105] LEE J Y, CHANG J K, DOMINGUEZ A A, et al. YAP-independent mechanotransduction drives breast cancer progression[J]. Nature Communications, 2019, 10: 1848.

[106] COSTE B, XIAO B L, SANTOS J S, et al. Piezo proteins are pore-forming subunits of mechanically activated channels[J]. Nature, 2012, 483(7388): 176-181.

[107] LIN Y C, GUO Y R, MIYAGI A, et al. Force-induced conformational changes in PIEZO1[J]. Nature, 2019, 573(7773): 230-234.

[108] 汝一雯, 张卫兵. 细胞力学信号转导中Piezo1机械敏感离子通道作用研究进展[J]. 中国细胞生物学学报, 2019, 41(8): 1622-1627.
RU Y W, ZHANG W B. Advances in the role of mechanosensitive ion channel Piezo1 in cellular mechanotransduction[J]. Chinese Journal of Cell Biology, 2019, 41(8): 1622-1627.

[109] TSE J R, ENGLER A J. Preparation of hydrogel substrates with tunable mechanical properties[J]. Current Protocols in Cell Biology, 2010, 47(1): 10.16.1-10.16.16.

[110] M JONKER A, A BODE S, H KUSTERS A, et al. Soft PEG-hydrogels with independently tunable stiffness and RGDS-content for cell adhesion studies[J]. Macromolecular Bioscience, 2015, 15(10): 1338-1347.

[111] DAVIRAN M, CATALANO J, SCHULTZ K M. Determining how human mesenchymal stem cells change their degradation strategy in response to microenvironmental stiffness[J]. Biomacromolecules, 2020, 21(8): 3056-3068.

[112] TAN J L, TIEN J, PIRONE D M, et al. Cells lying on a bed of microneedles: an approach to isolate mechanical force[J]. Proceedings of the National Academy of Sciences of the United States of America, 2003, 100(4): 1484-1489.

[113] GHASSEMI S, MEACCI G, LIU S M, et al. Cells test substrate rigidity by local contractions on submicrometer pillars[J]. Proceedings of the National Academy of Sciences of the United States of America, 2012, 109(14): 5328-5333.

[114] TRICHET L, LE DIGABEL J, HAWKINS R J, et al. Evidence of a large-scale mechanosensing mechanism for cellular adaptation to substrate stiffness[J]. Proceedings of the National Academy of Sciences of the United States of America, 2012, 109 (18): 6933-6938.

[115] SUN Y B, YONG K M A, VILLA-DIAZ L G, et al. Hippo/YAP-mediated rigidity-dependent motor neuron differentiation of human pluripotent stem cells[J]. Nature Materials, 2014, 13(6): 599-604.

[116] BAI T, SUN F, ZHANG L, et al. Restraint of the differentiation of mesenchymal stem cells by a nonfouling zwitterionic hydrogel[J]. Angewandte Chemie International Edition, 2014, 53(47): 12729-12734.

[117] HEO S J, SZCZESNY S E, KIM D H, et al. Expansion of mesenchymal stem cells on electrospun scaffolds maintains stemness, mechano-responsivity, and differentiation potential[J]. Journal of Orthopaedic Research, 2018, 36(2): 808-815.

[118] JAIN N, IYER K V, KUMAR A, et al. Cell geometric constraints induce modular gene-expression patterns *via* redistribution of HDAC3 regulated by actomyosin contractility[J]. Proceedings of the National Academy of Sciences of the United States of America, 2013, 110(28): 11349-11354.

[119] MCWHORTER F Y, WANG T T, NGUYEN P, et al. Modulation of macrophage phenotype by cell shape[J]. Proceedings of the National Academy of Sciences of the United States of America, 2013, 110(43): 17253-17258.

[120] NEVES S R, TSOKAS P, SARKAR A, et al. Cell shape and negative links in regulatory motifs together control spatial information flow in signaling networks[J]. Cell, 2008, 133(4): 666-680.

[121] RANGAMANI P, LIPSHTAT A, AZELOGLU E U, et al. Decoding information in cell shape[J]. Cell, 2013, 154(6): 1356-1369.

[122] ZHONG W L, ZHANG W G, WANG S Y, et al. Regulation of fibrochondrogenesis of mesenchymal stem cells in an integrated microfluidic platform embedded with biomimetic nanofibrous scaffolds[J]. PLoS One, 2013, 8(4): e61283.

[123] SAUER F, OSWALD L, DE SCHELLENBERGER A A, et al. Collagen networks determine viscoelastic properties of connective tissues yet do not hinder diffusion of the aqueous solvent[J].

Soft Matter, 2019, 15(14): 3055-3064.

[124] CHARRIER E E, POGODA K, WELLS R G, et al. Control of cell morphology and differentiation by substrates with independently tunable elasticity and viscous dissipation[J]. Nature Communications, 2018, 9: 449.

[125] YANG Z G, KOU S Z, WEI X, et al. Genetically programming stress-relaxation behavior in entirely protein-based molecular networks[J]. ACS Macro Letters, 2018, 7(12): 1468-1474.

[126] BALIKOV D A, NEAL E H, LIPPMANN E S. Organotypic neurovascular models: past results and future directions[J]. Trends in Molecular Medicine, 2020, 26(3): 273-284.

[127] BOEKHOVEN J, STUPP S I. 25th anniversary article: supramolecular materials for regenerative medicine[J]. Advanced Materials, 2014, 26(11): 1642-1659.

[128] PRIOR N, INACIO P, HUCH M. Liver organoids: from basic research to therapeutic applications[J]. Gut, 2019, 68(12): 2228-2237.

[129] SATO T, CLEVERS H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications[J]. Science, 2013, 340(6137): 1190-1194.

[130] SHANSKY J, DEL TATTO M, CHROMIAK J, et al. A simplified method for tissue engineering skeletal muscle organoids *in vitro*[J]. In Vitro Cellular & Developmental Biology Animal, 1997, 33(9): 659-661.

[131] BRAUNECKER W A, MATYJASZEWSKI K. Controlled/living radical polymerization: features, developments, and perspectives[J]. Progress in Polymer Science, 2007, 32(1): 93-146.

[132] ONG L L, HANIKEL N, YAGHI O K, et al. Programmable self-assembly of three-dimensional nanostructures from 10 000 unique components[J]. Nature, 2017, 552(7683): 72-77.

[133] ROSALES A M, ANSETH K S. The design of reversible hydrogels to capture extracellular matrix dynamics[J]. Nature Reviews Materials, 2016, 1: 15012.

[134] WANG R, YANG Z G, LUO J R, et al. B_{12}-dependent photoresponsive protein hydrogels for controlled stem cell/protein release[J]. Proceedings of the National Academy of Sciences of the United States of America, 2017, 114(23): 5912-5917.

[135] YANG Z G, YANG Y, WANG M, et al. Dynamically tunable, macroscopic molecular networks enabled by cellular synthesis of 4-arm star-like proteins[J]. Matter, 2020, 2(1): 233-249.

[136] ZHANG X L, DONG C M, HUANG W Y, et al. Rational design of a photo-responsive UVR8-derived protein and a self-assembling peptide-protein conjugate for responsive hydrogel formation[J]. Nanoscale, 2015, 7(40): 16666-16670.

[137] BAKER B M, CHEN C S. Deconstructing the third dimension: how 3D culture microenvironments alter cellular cues[J]. Journal of Cell Science, 2012, 125(Pt 13): 3015-3024.

[138] CALIARI S R, VEGA S L, KWON M, et al. Dimensionality and spreading influence MSC YAP/TAZ signaling in hydrogel environments[J]. Biomaterials, 2016, 103: 314-323.

[139] GERECHT S, BURDICK J A, FERREIRA L S, et al. Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells[J]. Proceedings of the National Academy of Sciences of the United States of America, 2007, 104(27): 11298-11303.

[140] HARRIS A K, WILD P, STOPAK D. Silicone rubber substrata: a new wrinkle in the study of cell locomotion[J]. Science, 1980, 208(4440): 177-179.

[141] OLIVER T, DEMBO M, JACOBSON K. Measurement of traction forces in cells locomoting along a substratum[J]. Cell Motility and the Cytoskeleton, 1998, 39(4): 342-344.

[142] DEL RIO A, PEREZ-JIMENEZ R, LIU R C, et al. Stretching single talin rod molecules activates vinculin binding[J]. Science, 2009, 323(5914): 638-641.

[143] RIEF M, GAUTEL M, OESTERHELT F, et al. Reversible unfolding of individual titin immunoglobulin domains by AFM[J]. Science, 1997, 276(5315): 1109-1112.

[144] GRASHOFF C, HOFFMAN B D, BRENNER M D, et al. Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics[J]. Nature, 2010, 466(7303): 263-266.

[145] CHEN B C, LEGANT W R, WANG K, et al. Lattice light-sheet microscopy: Imaging molecules to embryos at high spatiotemporal resolution[J]. Science, 2014, 346(6208): 1257998.

[146] GARAKANI K, SHAMS H, MOFRAD M R K. Mechanosensitive conformation of vinculin regulates its binding to $MAPK_1$[J]. Biophysical Journal, 2017, 112(9): 1885-1893.

通讯作者： 曹毅（1979—），男，教授，博士生导师。研究方向为单分子生物物理、蛋白质多肽生物材料、生物力学。

E-mail：caoyi@nju.edu.cn

第一作者： 宋成治（1998—），男。研究方向为实验生物物理和理论生物物理。

E-mail：857093988@qq.com