Summary. Embryonic development and differentiation are controlled largely by external stimuli. Mechanical forces, such as those exerted by the surrounding cells and tissues, gravity and substrate rigidity, have been shown to affect cell morphology and spreading, thus triggering signaling pathways that dictate their development. These mechanosignaling pathways play important roles in cellular differentiation and the determination of cell fate. In this review, we discuss the effects of external environmental stimuli on cell differentiation and how this affects pluripotency, as well as the key molecules and pathways involved in mechanosignaling, particularly in relation to embryonic stem cells. Advances in experimental techniques and devices used to study the different aspects of mechanobiology are also examined. Finally, the effects of mechanical stress on the initiation and maintenance of pathological processes such as cancer, as well as their implications for prognosis and possible therapies, are discussed.

Key words: Preimplantation development, Embryonic stem cells, Pluripotency, Mechanosignaling

Introduction

Mammalian development starts with fertilization, where highly specialized and differentiated haploid gametes, namely the sperm and the oocyte, fuse to generate a globular diploid zygote. Once fertilization is completed, the zygotic cell proliferates and undergoes dynamic epigenetic and chromatin reprogramming to gain totipotency (Reik, 2007), which enables these cells to differentiate into germ, somatic and placental cell lineages. This process is governed by numerous transcription factors acting in a concerted and time-dependent manner. One of the earliest events essential for this preimplantation developmental process is the activation of the pluripotency program by STAT3, which translocates to the nucleus to activate key transcription factors such as Oct4 and Nanog (Do et al., 2013). These pluripotency factors program the cells to become the pluripotent inner cell mass (ICM) (Nichols et al., 1998), which eventually generates the somatic cells constituting the fetal body.

Some mammals, such as rodents, can arrest their embryogenesis at the blastocyst stage if the surrounding environment is not suitable for pregnancy and/or birth. This phenomenon is known as delayed implantation or embryonic diapause, and the embryo remains unimplanted in the uterus until the environment is more favorable for its further development. However, once the embryo is implanted into the uterine wall, the cells undergo rapid differentiation into the placental tissue and fetal body. This suggests that pluripotency can be maintained if the embryo is prevented from implanting.

Recent findings have shown that the application of...
mechanical force is essential for implantation of the embryo into the uterine mucosa as well as for defining the embryonic axis of the embryo (Hiramatsu et al., 2013). These forces from external stimuli may have a direct impact on inducing differentiation and conferring protection, thus contributing to the maintenance of pluripotency in the embryo (Chowdhury et al., 2010; Chan et al., 2013).

Mouse embryonic stem cells (mESCs) have been extensively utilized as model systems for the study of pluripotency. ESCs are originally derived from the ICM cells of blastocysts (Evans and Kaufman, 1981; Martin, 1981) and can be propagated indefinitely in culture media supplemented with leukemia inhibitory factor (LIF) and serum. They have the ability to differentiate into all three germ layers (the endoderm, mesoderm and ectoderm) both in vitro and in vivo, and are further characterized by their highly dynamic and plastic nucleus (Meshorer et al., 2006; Boskovic et al., 2014; Ueda et al., 2014), which enables homologous recombination to occur with higher efficiency compared to other cell types. These features uniquely position ESCs as invaluable tools for mouse genetic studies through the generation of genetically modified mice, and have advanced our knowledge in developmental biology and disease studies enormously in the past few decades (White et al., 2013).

The relationship between cell shape and pluripotency is well accepted but it has not been fully investigated. For instance, it is common knowledge for researchers working with ESCs that cells with a flattened shape are differentiated cells, and are not suitable for the production of chimeric mice. Conversely, undifferentiated cells are characterized by their roundness and their ability to form tight and compact dome-shaped colonies in culture. The mechanisms linking cell shape and pluripotency are not yet fully elucidated, but researchers who work on mouse developmental engineering still empirically inject round ESCs into host embryos to generate chimeric mice.

Accumulating evidence suggests that cell shape, which reflects mechanical stress (including gravity) from the surrounding environment, has a direct impact on the differentiation status of the cells. Wakayama et al. analyzed mammalian fertilization and preimplantation development in microgravity conditions using a three-dimensional clinostat, and found that microgravity caused slower development and fewer trophectoderm cells compared to the control 1G condition (Wakayama et al., 2009). They reported that microgravity had a positive effect on the expression of pluripotency markers while having a negative effect on those of differentiation. In an ES cell study, Ying et al. established a method called 2i, a combination of small molecule inhibitors of the mitogen-activated protein kinase (MAPK) pathway and glycogen synthase kinase 3 (GSK3), to sustain pluripotency in ESCs independent of feeder cells, serum and cytokines. 2i-cultured ESCs are characterized by the ability to form almost perfectly round colonies with very loose attachment to the feeder cells or culture dish, compared to the ESCs cultured in serum and LIF (Fig. 1).

Furthermore, through live-cell imaging analyses of the mESC derivation process, we have previously reported that the expression of pluripotency marker Oct4 was lost when pluripotent epiblast cells were stretched (Yamagata et al., 2010). These findings led us to further investigate the relationship between mechanosignaling and pluripotency. In this review, we discuss the signaling pathways linking cell shape and pluripotency, the techniques that are currently being used to study this phenomenon and the pathological conditions that may arise from the disruption of these pathways.

**Key molecules and pathways governing mechanotransduction**

All creatures on Earth are subjected to the effects of gravity, and numerous physical forces are continuously generated and received when we move. These multiple forces influence the functions of the heart, blood vessels, respiratory organs, bones and other tissues. To respond
to such physical stimuli, our body has developed mechanosensory receptors, which allow it to detect and adapt to the physical environment. When physical stimuli are externally applied to tissue or cultured cells, the mechanical stress experienced by the cells is detected by the focal adhesion points located on the cell membrane and turned into intracellular signaling in a process called mechanotransduction, which allows the cells to respond to these stimuli. Here, we will look at some of the key molecules and pathways involved in mechanosignaling.

Focal adhesion complexes occur at the focal adhesion points and mechanically link the extracellular matrix (ECM) to the cell, acting as key platforms for mechanotransduction. They consist of the integrins which bind to the ECM and are the points of contact the cell has with the extracellular environment, adapter proteins which link the integrins to the cytoskeleton of the cell, and finally the intracellular signaling proteins which are the downstream effectors of intracellular signaling. Physical information can be transduced into intracellular biochemical signals through conformational changes of focal adhesion-related proteins, such as p130Cas (Sawada et al., 2006), talin (del Rio et al., 2009), vinculin (Grashoff et al., 2010), and the integrins themselves (Friedland et al., 2009). Exogenous force can stimulate the integrins (Tzima et al., 2001), causing the modulation of focal adhesion components (Riveline et al., 2001) and activating downstream signaling molecules, such as focal adhesion kinase (FAK) (Guan, 1997) and the Rho family of small GTPases (Geiger et al., 2009) (Fig. 2).

FAK is one of the key factors in mechanotransduction. Being a kinase, it is activated by phosphorylation, which occurs at its Y397 residue. FAK autophosphorylation promotes the recruitment and activation of Src kinases and itself to form the FAK–Src signaling complex. This complex mediates integrin-dependent cytoskeletal reorganization (Shi and Boettiger, 2003), thereby influencing cell growth, viability, differentiation, and motility (Lo et al., 2000; Engler et al., 2004). Chowdhury and colleagues reported that stress-induced ES cell-spreading depends on myosin II activity, Src and Cdc42 (Chowdhury et al., 2010). This was further supported by the blocking of stress-induced ES cell spreading with PP1, a specific Src tyrosine phosphorylation inhibitor (Chowdhury et al., 2010). Src is thus a critical molecule in the initiation of stress-induced spreading as well as in the spontaneous early spreading of adherent cells (Chowdhury et al., 2010) (Fig. 2), consistent with our results which show that the activation of FAK–Src and MAPK pathways is highly correlated with the mechanical induction of mESC spreading (Shimizu et al., 2012).

The Rho family of GTPases is known to influence cell morphology, and is responsible for the formation of actin stress fibers, lamellipodia, and filopodia through the regulation of the focal adhesion complexes (Nobes and Hall, 1995). Rho activity is influenced by matrix compliance/stiffness (Wozniak et al., 2003), which in turn modulates cytoskeletal tension. Mcbeath et al. have demonstrated that human mesenchymal stem cells (MSCs) undergo adipogenesis or osteogenesis depending on whether cells are rounded or allowed to spread (McBeath et al., 2004). This lineage commitment is dependent on mechanical cues from the cells' environment, such as the substrate they are on, and is mediated by RhoA. Rho-mediated cytoskeletal tension promotes focal adhesion assembly (Burridge and Wennerberg, 2004) and induces ERK-dependent growth and cell migration via actomyosin contractility (Chrzanowska-Wodnicka and Burridge, 1996) (Fig. 2). Taken together, cell behavior, such as cell migration (Murali and Rajalingam, 2014), is functionally related to matrix stiffness, Rho activity and cell contractility.

When mechanical stress is detected at the focal adhesion points, these signaling pathways are activated, eventually leading to the polymerization of actin filaments and the formation of actin bundles. It has been reported that the actin polymerization at focal adhesions is controlled by several actin nucleation or elongation factors, such as diaphanous-related formins, including Dia1 and Dia2 (Gupton et al., 2007) and the Ena/vasodilator stimulated phosphoprotein (VASP) family of proteins (Hirata et al., 2008). Myosin II interacts with these actin filament bundles, generating mechanical force to contract the bundles, leading to morphological changes and/or the initiation of migration (Aguilar-Cuenca et al., 2014).

Mechanical stimulation has been shown to modulate the morphology and behavior of various types of cells (Katsumi et al., 2002; Wu et al., 2007; Adamo et al., 2009). In addition to the mechanosensing adaptor proteins, it is known that mechanosensitive (MS) channels, including Ca2+-permeable channels, are

![Fig. 2. Key molecules and pathways governing mechanotransduction.](image-url)
mechanotransducers. The stereocilia of hair cells in the inner ear are one such example, and contribute to hearing and balance via the gating spring theory, whereby the mechanical stress of sound waves on the stereocilia causes activation of mechanically-gated ion channels in the hair cells, translating physical stimuli into chemical intracellular signaling and ultimately electrical signaling to the auditory nerve (Ricci et al., 2006). A recent report demonstrated that MS channel activation occurred adjacent to focal adhesions after mechanical stimulation, suggesting that the MS channels may interact with adhesion molecules, forming a mechanosensing molecular complex (Hayakawa et al., 2008).

Together, these molecules and pathways highlight the important role of mechanotransduction in cell development and adaptation with respect to the cellular environment and extracellular signals.

Mechanobiological techniques and their applications

Research in the field of cell biology has led to the development of multiple techniques to study mechanotransduction and cell mechanics in vitro, and these in turn have further advanced our knowledge. In this section, we describe key techniques that have been used in mechanobiology studies.

Cell stretching has been known to activate mechanotransduction (Geiger et al., 2009). To analyze how mechanotransduction can be activated by cell stretching, cells can be seeded on silicone-coated substrates, which can be stretched uniaxially or biaxially (Sawada and Sheetz, 2002; Morioka et al., 2011). Uniaxial cyclic stretching induces cell alignment and cytoskeletal reorganization caused by the microtubule-stress fiber assembly, resulting in the maintenance of homeostatic strain on the stress fibers at focal adhesions (Morioka et al., 2011).

Mechanical properties of the ECM are crucial factors in the regulation of the cellular cytoskeleton and cell morphology. These mechanical properties are able to influence the expression of cytoskeleton proteins such as F-actin (filamentous actin), thereby affecting cell shape. F-actin staining is thus often used to determine the amount and pattern of stress fibers in the cell. Singhvi et al. showed that cells cultured on small square islands of 20×20 μm with a patterned adhesive surface displayed punctate F-actin distribution with few stress fibers. When these cells were cultured on 50×50 μm domains, the number of stress fibers increased in proportion to the expansion of domain and hence cell area attached to the substratum. Furthermore, when stress fibers were disrupted by cytochalasin D treatment, the cells maintained their original shape, indicating that the observed stress fibers developed downstream of cell morphology. The use of microdomains of different shapes further enables the elucidation of the relationship between cell shape and function. For example, it was reported that cell shape is a critical determinant that switches cell fate between death and proliferation via geometric switching between apoptosis and DNA synthesis modulated by ECM-integrin-dependent mechanical signals (Chen et al., 1997).

During development and tissue repair, progenitor cells are controlled by topographical signals. These topographical cues have been shown to play important roles in determining cell fate. By culturing cells on topographical structures of different geometries and sizes under identical experimental conditions, cell-topography interactions and their effect on cell shape and size can be elucidated. To analyze the precise spreading requirements for cellular function, cells can be cultured on different-sized adhesive islands (Chen et al., 1997). When cells were plated on square-shaped islands, these cells grew in a manner that closely matched the size and shape of the adhesive island (Chen et al., 1997). The multi-architecture chip (MARC) can be employed to investigate the influence of different geometries and sizes in the substrate topography on cell fate. The MARC contains an array of distinct topographies which allows the simultaneous observation of cells grown on different topographies in identical conditions, facilitating high throughput studies of cell-topography relationships and interactions (Moe et al., 2012). In a study of neural differentiation, human embryonic stem cells (hESCs) were subjected to differentiation by culturing small aggregates of undifferentiated hESCs without embryoid body formation on the MARC in a mixture of N2 and B27 supplements for 7 days. Through this study, it was found that anisotropic patterns, such as gratings, promoted neuronal differentiation while isotropic patterns, such as pillars and wells, promoted glial differentiation of hESCs, demonstrating that the interplay between topography and biochemical cues has a direct impact on differentiation.

To investigate how cells respond to the mechanical properties of the cellular environment, such as the elasticity of the substrate, cells can be cultured on substrates of different elasticities. Substrate stiffness influences the cell-adhesion strength and the degree of cell spreading. Polyacrylamide gel is often used in such studies as the bis-acrylamide crosslinker concentration in polyacrylamide gel can be easily modified to adjust the substrate stiffness of the gel. Different substrates, such as Type I collagen, can then be covalently linked to the gel surface using the crosslinking reagent N-Sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino) hexanoate (Sulpho-SANPAH) (Pelham and Wang, 1997). The matrix stiffness can directly cell morphology and lineage specification of MSCs (Engler et al., 2006). Using a well-defined, elastically tunable gel system coated with collagen, human MSCs were shown to differentiate into neurons, myoblasts or osteoblasts, depending on substrate elasticity. Soft matrices that mimic the texture of brain tissue induced neurogenic differentiation, whereas stiffer matrices that mimic that of muscle tissue produced myogenic cells, and...
comparatively rigid matrices that mimic the hardness of collagenous bone induced differentiation into osteogenic cells (McBeath et al., 2004). The expressions of key lineage markers and myosins have been found to be dependent on the matrix elasticity. In particular, the expression of the contractile protein non-muscle myosin II has been shown to be dependent on the level and activity of its transcription factor MyoD1, which is in turn regulated by matrix elasticity (Engler et al., 2006). As myosins are heavily involved in the determination of cell shape and behavior, the regulation of myosin protein expression by substrate elasticity provides an important link to validate the influence of the ECM on cell morphology.

**Mechanosignaling pathways leading to differentiation**

Recent studies demonstrated that there are two distinct states of pluripotency during development, i.e. the ground and primed states (Brons et al., 2007; Tesar et al., 2007; Nichols and Smith, 2009; Hanna et al., 2010). Uterine implantation causes rodent epiblasts to undergo morphological transformation into a cup-shaped structure known as the egg cylinder (Nichols and Smith, 2009), and stem cells derived from this epiblast, so called epiblast stem cells (EpiSCs), are in the primed state and have restricted pluripotency compared to those derived from the ICM, which retain the ability to differentiate to germ cells. While uterine implantation appears to provide a cue to the conversion from the unrestricted to restricted pluripotency of stem cells, the underlying mechanism remains to be revealed.

The difference in pluripotency of EpiSCs and cells from the ICM implies that it is not only the action of cytokines but also the elasticity of the extracellular matrix, such as that of the uterus during uterine implantation, that may regulate the transition of stem cells from the ground state to the primed state in vivo. Huang et al. have shown that the surface on which the cell is on exerts tension on the actin cytoskeleton of the cell and induces cell distortion, which triggers various cell signaling pathways that allow the cell to proliferate, enter quiescence, differentiate or undergo apoptosis in response to the environmental conditions (Huang and Ingber, 2000). While cytokines play crucial roles in the maintenance of pluripotency and/or induction of differentiation of stem cells, we now know that the surrounding mechanical environment also plays important roles. Matrix stiffness tends to be more effective and selective than cytokines in inducing MSC differentiation (Engler et al., 2006). Thus, it may be possible that implantation alters the mechanical environment of the epiblast, including substrate elasticity.

Cell function and differentiation have been reported to be regulated by substrate stiffness, and are therefore regulated by the mechanical properties of the tissue where the cells are located (Engler et al., 2004). As described previously, human MSCs have been reported to commit to their lineage depending on the substrate elasticity and actomyosin contractility (Engler et al., 2006). Likewise, gene expression analyses demonstrated that the expression of pluripotency and differentiation markers were dysregulated in mESCs depending on the elasticity of the substrate on which they were cultured. mESCs, in contrast to fibroblasts which typically spread over a greater area on stiffer substrates than on softer substrates, do not exhibit extensive spreading on stiff substrates, suggesting a difference in force sensing or responsive machineries between ESCs and other cell types (Yeung et al., 2005).

It is important to analyze if the elasticity of the surrounding microenvironment during epiblast differentiation falls into the range that allows substrate elasticity-induced differentiation of mESCs. The elastic moduli of normal tissues lie in the range of 1 - 10 kPa (Jannmey and McCulloch, 2007). We found that mESCs cultured on a 7.5 kPa substrate had decreased expression of pluripotency markers, such as Oct4 and Sox2, compared with those grown on a 67 kPa substrate. In contrast, the expression of the differentiation markers for mesodermal and endodermal cell lineages, such as Gata6 and Foxa2, was significantly higher in mESCs grown on the 7.5 kPa substrate without LIF (Shimizu et al., 2012). Chowdhury and colleagues also reported that after continuous application of local stress by magnetic beads (17.5 Pa at 0.3 Hz for 60 min), the Oct4 expression of mESCs was downregulated by 35% within 24 hours, and by 50% within 72 hours, suggesting that local cyclic stress detected through focal adhesions might also be sufficient to diminish Oct4 expression in mESCs and induce their differentiation (Chowdhury et al., 2010). Interestingly, when mESCs were cultured in suspension culture, cells expressed higher levels of pluripotency markers and lower levels of differentiation markers compared to adherent cells (Andang et al., 2008). This may indicate that releasing ESCs from mechanical stress, including that exerted by a firm substrate, may allow easier maintenance of pluripotency in culture.

Activation of the FAK-Src and MAPK signaling pathways is involved in the mechanical induction of mESC differentiation. Loss of pluripotency in mESCs on substrates with high elasticity was closely correlated with enhanced activity of FAK, Src and MAPK (Shimizu et al., 2012). Consistent with previous reports describing MAPK as being downstream of Src in osteoblasts (Boutahar et al., 2004), the Src kinase inhibitor CGP777675 attenuated the substrate elasticity-dependent activation of MAPK (Shimizu et al., 2012). With regard to the role of Src-MAPK signaling in the regulation of pluripotency, it was reported that the inhibition of MAPK-kinase results in the intranuclear accumulation of Tbx3, a protein that sustains the expression of Oct4 (Niwa et al., 2009). ERK/MAPK signaling has also been implicated in the regulation of pluripotency through substrate elasticity. It was discovered that cells grown on
polyacrylamide of low elastic modulus (0.5 kPa) could not form stable focal adhesions, leading to reduced focal adhesion formation and reduced ERK/MAPK signaling (Trappmann et al., 2012). This report is in good agreement with our results that the differentiation of mESCs is closely related to the activation of focal adhesion kinase and ERK/MAPK signaling (Shimizu et al., 2012).

As described previously, the pluripotency of mESCs can be sustained in a defined medium with the dual inhibition (2i) of the MAPK ERK1/2 and GSK3 signaling pathways, with the mESCs displaying the characteristic compact colony morphology of undifferentiated pluripotent cells (Ying et al., 2008) (Fig. 1). However, the expressions of Nanog and Oct4 tend to be decreased in the 2i-treated mESCs on softer substrates of between 2 and 7.5 kPa (Shimizu et al., 2012). Interestingly, these elasticities coincide with the elastic moduli of normal tissues, which lie in the range of 1 to 10 kPa (Janmey and McCulloch, 2007). Corresponding to the significant involvement of Src in the mechanical regulation of cellular differentiation, we hypothesized that the inhibition of Src signaling may complement the conventional 2i method to support the maintenance of ESC pluripotency in a particular mechanical microenvironment. Indeed, the dual inhibition of GSK3 and Src (alternative 2i), instead of GSK3 and MAPK, sustained the pluripotent phenotype of mESCs (Shimizu et al., 2012), with almost all mESC colonies remaining morphologically undifferentiated under the alternative 2i treatment. Most importantly, this alternative 2i treatment with the addition of LIF was highly efficient in deriving germline competent ESCs from mouse preimplantation embryos, indicating that stem cells can be controlled by modulating mechanosignaling pathways.

These results implicate the physical effects of the microenvironment on stem cell differentiation and these pathways provide potential avenues and targets for the development of novel regenerative therapeutics against diseases involving stem cell differentiation.

**Cell shape and pathological processes**

Pathologists have known for decades that cancer patients with more undifferentiated cells in their tumors tend to have poor prognosis. Transcriptomic analyses of cancer cells have shown that malignant cells have gene signatures similar to that of ESCs (Widschwendter et al., 2007; Ben-Porath et al., 2008). Stem cell-like properties have also been reported to increase malignancy of cancer cells, promoting tumorigenesis, metastasis and resistance to cancer therapies (Liao et al., 2014). However, it is unknown whether the cancer cells attain these ES cell-like signatures as they become more malignant, or if these ES cell-like qualities themselves confer malignancy to these cancer cells in the first place. Despite the large number of studies looking at the genetic similarities between malignant cancers and ESCs, little attention has been given to how similarities in cell shape between these malignant cancer cells and ESCs might contribute to the poorer prognosis of cancer patients with more undifferentiated cancer cells.

It is known that cell shape plays an important role in cell growth. The growth of untransformed cells is affected by their ability to attach to the substrate and their confluence, which in turn determine how much the cells spread and their resulting shape. Cells which are more confluent or are not able to attach well to the substratum are spheroidal in shape and have been shown to have decreased DNA synthesis (Folkman and Moscona, 1978), reflecting slower rates of cell proliferation. When normal cells are detached from the substratum, they undergo a type of programmed cell death known as anoikis. This occurs due to the loss of signals from the ECM and neighboring cells, which are essential for cell growth and survival (Frisch and Francis, 1994). In contrast, transformed cells are resistant to anoikis and are able to continue proliferating despite high cell confluence and the lack of anchorage, indicating that they are able to overcome the inhibitory effects of cell crowding and anchorage-dependent growth, and can survive without signaling from the ECM and other cells. These cells are able to continue proliferating, even when detached from the substratum, in a spheroid. In fact, this process is called epithelial-mesenchymal transition (EMT) and confers a survival advantage to these transformed cells, increasing their malignancy (Paoli et al., 2013). Populations of cancer cells with the ability to form spheroids have been identified, and it is believed that these cells are tumor initiating cancer stem cells (Cao et al., 2011).

EMT is a process whereby adherent epithelial cells lose their apico-basal polarity and undergo drastic changes in cell shape to become mesenchymal cells, which are able to proliferate without the need to be attached to the basal lumina (Thiery et al., 2009). During embryonal development, EMT is required to generate different cell types of specialized functions from the epithelium. However, this process can also occur in cancer to greatly increase the ability of malignant cells to migrate and invade into other organs and tissues, thus facilitating metastasis. Vimentin, known to be highly expressed in mesenchymal cells, has been shown to be responsible for the changes in cell shape during EMT, making epithelial cells adopt a more spherical mesenchymal shape (Mendez et al., 2010). The changes in cell shape are accompanied by increased cell motility and decreased cell adhesion. Vimentin has also been shown to contribute to the invasiveness and aggressiveness of cancer (Lang et al., 2002; Vuoriluoto et al., 2011). Conversely, loss of E-cadherin, associated with epithelial cells, can cause cell dedifferentiation into a more mesenchymal phenotype with changes to cell morphology, motility and invasiveness (Fri xen et al., 1991; Vlemingkx et al., 1991; Handschu h et al., 1999).

Differentiation can also be triggered when cells are prevented from attaching to a surface. Watt et al. showed
that when human epidermal keratinocytes were prevented from attaching and were kept spherical, DNA synthesis was inhibited and involucrin, a marker of terminal differentiation, was prematurely expressed (Watt et al., 1988). This forced differentiation may also contribute to the lower rates of DNA synthesis and proliferation observed in detached cells as previously mentioned. Despite being able to undergo EMT to detach from the substrate and metastasize to other sites, it has been shown that poorly differentiated breast cancer cell lines are more invasive and malignant (Sommers et al., 1994). It is thus possible that the ability of cancer cells to undergo EMT and metastatize is due to aberrations in the mechanosignaling pathways involved in forcing differentiation in detached cells.

Tumor rigidity likely reflects an elevation in invasive tissue pressure and solid stress due to tumor expansion (Padera et al., 2004). An increase in the elastic modulus of transformed cells is mediated by changes in the composition of the tumor tissues and may enhance tumor metastasis (Akiri et al., 2003). Whether tissue stiffness can actively promote malignant transformation has yet to be assessed. As Rho activity is often elevated in stiff tumors (Fritz et al., 1999), leading to the activation of downstream ROCK and increasing tumor metastasis (Croft et al., 2004), it is possible that tissue stiffening may drive transformation by Rho-dependent cytoskeletal tension, leading to a more malignant phenotype of tumors through force-dependent regulation of the integrins. Indeed, integrins and Rho GTPases have been shown to modify the tumorigenic behavior of a tissue (White et al., 2004), and integrin levels and signaling are altered in stiff tumors (Guo and Giancotti, 2004). These reports suggest that tissue stiffness could drive the malignant behavior of tumors through Rho-dependent integrin modulation.

Targeting of mechanosignaling pathways leading to differentiation may be a possible approach for cancer therapy. Normal somatic cells experience slowed growth due to contact inhibition and the pressure from neighboring cells when they become confluent. By inducing mechanosignaling pathways leading to cellular differentiation, it may be possible to inhibit both uncontrolled growth and the ability of these transformed cancer cells to grow without signaling from the ECM. Bisanz et al. have shown that disrupting the ECM-integrin interaction in PC3 prostate cancer cells could inhibit growth and increase apoptosis in these cells (Bisanz et al., 2005). Besides inducing the mechanosignaling pathway between the host stroma and tumor cells, this signaling pathway can also be used to our advantage. In a review paper by Ingber, he discussed the possibility of ‘rebooting’ cancer cells by remodeling the ECM with exogenous scaffolds, causing the cells to revert to a normal non-malignant phenotype or to regenerate normal tissue (Ingber, 2008).

Mechanobiology research is becoming an increasingly important field for the understanding of the in vivo effects of mechanical stress to cell and organ properties, and is essential for the development of therapeutic approaches to diseases associated with mechanical stress in the cellular microenvironment. In order to further develop therapies based on the biomechanical signaling between the ECM and cells, much more has to be done to elucidate the molecular pathways involved and identify potential targets we can inhibit or activate.

Conclusion

Studies exploring the relationship between mechanical stress and stem cell properties are relatively new and how mechanosignaling is controlled in vivo is of particular interest. It is evident that external forces are able to trigger signaling pathways that influence cell differentiation and affect pluripotency. This mechanotransduction is mainly carried out by focal adhesion complexes, which connect the cell to the surrounding ECM and facilitate the transmission of mechanical stimuli along major pathways such as the MAPK pathway. The use of ESCs and various other cell types to study this phenomenon has been crucial to the advancements made in the field of mechanosignaling. Numerous techniques have been developed and employed to better study mechanosignaling, including the staining of major components of the focal adhesion complex and the culturing of cells on surfaces of varying textures, shapes, hardness, and stretchabilities. These have been used to elucidate cell-topography interactions and their influence on cell growth and behavior, and have even been further refined to increase the efficiency of such studies in high throughput arrays.

Despite the advances in understanding mechanosignaling, the mechanosignaling interaction with other cellular processes is still unclear, and more studies need to be carried out to fully elucidate these pathways. Although new techniques have advanced our knowledge on mechanobiology in vitro, there is still a pressing need to develop more sophisticated devices and methods to monitor and control mechanical stresses accurately in vivo. This is essential in the management of diseases such as cancer, as their onset and prognosis may be influenced by mechanical stimuli. There is also increasing evidence that cell shape may be indicative of disease aggressiveness. In particular, a spheroidal cell shape may increase proliferation rates, induce EMT and enhance metastasis, all of which are associated with more malignant and invasive cancers. However, as much is yet unknown about the link between cell shape and disease occurrence, it is imperative that this aspect of mechanosignaling is further studied to better understand the underlying molecular mechanisms. The knowledge of these mechanisms applied with the manipulation of the external environment as well as the interference in mechanosignaling pathways may thus prove to be a potential therapeutic approach to combating cancer and
other diseases.

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Cell shape and pluripotency


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