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STEM CELL MECHANOBIOLOGY AND ITS ADVANCE IN THE STEM CELL THERAPY AND CANCER RESEARCH

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ABSTRACT

Stem cells are undifferentiated cells that are capable of proliferation, self-maintenance, and differentiation towards specific cell phenotypes. These processes are controlled by a variety of cues including physicochemical factors associated with the specific mechanical environment in which the cells reside. The control of stem cell biology through mechanical factors remains poorly understood and is the focus of the developing field of mechanobiology. An understanding of the cytoskeleton's importance in stem cells is essential for their manipulation and further clinical application. The cytoskeleton is crucial in stem cell biology and depends on physical and chemical signals to define its structure. Stem cells and their local microenvironment, or niche, communicate through mechanical cues to regulate cell fate and cell behavior and to guide developmental processes. Potential stem cell therapy holds great promise for the treatment of many diseases such as stroke, traumatic brain injury, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, myocardial infarction, muscular dystrophy, diabetes, etc. It is generally believed that transplantation of specific stem cells into the injured tissue to replace the lost cells is an effective way to repair the tissue. In fact, organ transplantation has been successfully practiced in clinics for liver or kidney failure. The study of physical and mechanical features of cancer cells, especially mechanisms associated with cancer cell invasion and metastasis, may help the effort of developing diagnostic biomarkers and therapeutic drug targets.

KEYWORDS: Embryonic Stem Cell; Mesenchymal Stem Cell; Mechanobiology; Cell Mechanics; Differentiation; Extracellular Matrix.

1. INTRODUCTION

Stem cells are undifferentiated cells that are capable of proliferation, self-maintenance, and differentiation towards specific cell phenotypes [Morrison et al., 1997; Reya et al., 2001]. The possession of these features makes stem cells an attractive source for cell-based regenerative therapies. However, the success of such therapies is dependent on the understanding, and ultimately the control of the processes of both self-maintenances, required to maintain their "stem-ness" and also lineage-specific differentiation. Self-maintenance and differentiation of stem cells in vivo are controlled by a microenvironment described as the 'stem cell niche', which has been defined as a specific location in a tissue where stem cells reside for an indefinite period of time and produce progeny cells while self-renewing [Ohlstein et al., 2004]. Cells that are no longer retained within the niche environment may undergo differentiation, which is also controlled by factors associated with their new microenvironment.

Mechanobiology is defined as the study of mechanisms by which cells detect and respond to mechanical stimuli. The mechanical forces imposed on the growing and differentiating fetal tissues play an increasingly critical role in the developmental process, effectively guiding the development of the functional organism. Post-partum, mechanical demands increase dramatically. As such major morphological changes progressively unfold under the influence of the mechanical stimuli provided by external gravitational forces and muscular contraction. Indeed, mechanical forces have been reported to affect developmental processes as diverse as gastrulation in *Drosophila*. Three major contributory factors may be involved. First direct cell-generated forces are associated with the contractile components of the cytoskeleton and associated linkages to the extracellular matrix. Secondly, the ability of cells to detect and respond to the stiffness of their surrounding environment, via durotaxis, and finally, the effect of external mechanical forces, generated via gravitational effects, muscle action,or other cell contractile processes. These processes do not work in isolation and the mechanical environment of stem cells may involve contributions from all three processes in a

highlycomplex and interacting manner. Mechanobiology contributes first to the understanding of the hierarchical mechanical properties at the tissue, cellular and subcellular levels and the inter- relationship betweenthese properties. Moreover, it reveals fundamental processes associated with development, normal physiology, and pathology, through elucidation of mechanotransduction pathways, by which mechanical perturbation is transduced into a biological response.

The mechanical properties of stem cells influence their response to the mechanical environment, their ability to migrate, and ultimately their differentiation. Those studies attempting to characterize the mechanical properties of stem cells and their intracellular components, tend to utilize a short-term loading approach, often coupled with live-cell microscopy techniques. Many of these studies involvethe application of a mechanical force to an individual cell. However, to investigate the influence of mechanical forces on stem cell metabolism and differentiation, separate model systems are required involving prolonged culture in the presence of mechanical perturbation. These studies often employ a population of cells cultured either in a monolayer or within a 3D scaffold. This enables the examination of the heterogeneity of any response, due to the possible presence of subpopulations of cells and also spatial differences in the mechanical environment, such as localized differences in strain or fluid flow. Both single-cell and population approaches may be used to examine the underlying mechanotransduction signaling behavior in stem cells.[1]

Embryonic stem cells (ESCs) are isolated from the blastocyst and have the potential to generate any kind of cells from the three germ lines: ectoderm, mesoderm, and endoderm. Adult stem cells or somatic stem cells also referred to as tissue-specific stem cells, can be obtained from already born animals and humans, not necessarily adults, because infants also have adult stem cells. These stem cells are necessary to maintain the body during its lifetime, with a self-renewing capability but withoutthe potency to generate cells from the three germ lines.

Mesenchymal stem cells (MSCs) are a type of adult stem cells that is self-renewing and pluripotent.MSCs have the capacity to differentiate into several lineages, mainly adipocytes, chondrocytes, and osteocytes. On the other hand, hematopoietic stem cells (HSCs), another kind of adult stem cell, havethe potential to generate blood cells like lymphocytes, dendritic cells, natural killer cells, monocytes, and others, while neural stem cells (NSCs) can generate lineages from the nervous system, neurons, and glia (astrocytes and oligodendrocytes). Stem cells are present inside different types of tissue.

They have been found in tissues, including the brain, bone marrow, blood and blood vessels, skeletal muscles, skin, and liver.

Cancer stem cells (CSCs), also known as “cancer stem-like cells” or “tumor-initiating cells” (TICs) are a kind of stem cells that may express surface markers present on human ESCs and/or adult stem cells [8]. These cancer cells share the same properties of self-renewal and differentiation with stem cells, and for that reason are included in this category.

Biophysical aspects, such as the role of the actin cytoskeleton-mediated mechanobiology of stem cells, are important to consider. The conjunction and comprehension of these aspects present a better way for establishing all the possible interactions between biomaterials, cells, and organisms in orderto be used for medical purposes. The ability to design biomaterials to mimic natural scaffolds is a novel perspective for improving ordeveloping more efficient stem cell-based therapies in regenerative medicine, requiring a deep understanding of stem cell biology.[2]

Cancer is a deadly disease mainly because of its invasive and metastatic ability, which may be associated with a mechanotypical change in individual cancer cells as well as the changes in the cancer microenvironment. In recent years, emerging evidence has shown that cell mechanotypical changes, such as alterations in cellular stiffness and deformability, are common phenotypic events incancer development and progression.[7]

2. Stem cell mechanics - single-cell approaches Micropipette Aspiration

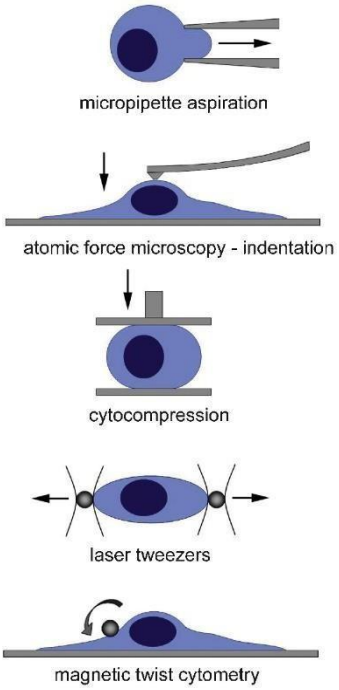

Fig. 1:

The micropipette aspiration technique is used to deform an individual cell with a known aspiration pressure (see Fig. 1). Such a technique has been widely used to determine the mechanical properties of a variety of cell types including stem cells [Hochmuth, 2000].

Micropipettes, with inner diameters typically between 5 and 15 mm, may be used to induce deformation of the whole cell, or alternatively discrete regions of the cell, depending on the cell diameter. Moreover, the properties of subcellular components, most notably the nucleus and cytoskeleton may be investigated. With the control of a hydraulic micromanipulator, the micropipette is moved into contact with the cell surface and pressures applied and the cell is visualized using bright field or fluorescence microscopy.

Two approaches have been used for performing the aspiration technique. For the incremental approach, pressure is appliedtypically insteps up to 5 cmH20 (0.49 kPa), with the equilibrium cell aspiration length, L, recorded at each pressure. The apparent young's modulus may then be determined using a theoretical model [There et al., 1988]. In this model, the cell is assumed to be a homogeneous, elastic half-space material and young's modulus, E, is therefore given as follows, where F(h) is defined as the wall function with a typicalvalue of 2.1:

$$E = \frac{3\phi(\eta)}{2\pi}\left(\frac{\Delta P}{L/a}\right) \quad (1)$$

The Young's modulus can thus be determined from the slope of the linear regression of the normalized length L/a versus the negative suction pressure P. For the alternative approach, in which pressure is applied in a single step, the following equation is fitted to the temporal changes in aspiration length measured experimentally (Eq. 2). This model assumes that the cell behaves as a homogenous linear viscoelastic three-parameter solid half-space.

$$L(t) = \frac{\phi(\eta) a \Delta P}{\pi k_1}\left(1 - \frac{k_2}{k_1 + k_2} e^{-t/\tau}\right) \quad (2)$$

The pipette internal diameter is given by 'a whilst the relaxation time constant 't' is defined as follows(Eq. 3).

$$\tau = \frac{\mu(k_1 + k_2)}{k_1 k_2} \quad (3)$$

The parameter k1 is termed the equilibrium or relaxation modulus (Er or E1), k1 þ k2 is theinstantaneous modulus (Ei) and m is the apparent viscosity. Using this viscoelastic model, it is also possible to determine the apparent equilibrium of young's model given by:

$$E = \left(\frac{3}{2}\right) k_1 \quad (4)$$

It should be recognized that although these models benefit from being simple, they neglect geometrical factors, such as finite cell dimensions, the evolution of the cell-micropipette contact region andcurvature of the micropipette edges. Thus, other models incorporating these geometric factors into acomputational form have been developed [Haider and Guilak, 2000], which can also account for theinhomogeneities in the cellular properties.[1]

3. The Role of Cytoskeletal Proteins in Stem Cells

Microfilament actin, intermediate filaments, and microtubules are three types of cytoskeleton proteins that exist in all cell types. These have all been implicated in cancer cell mechanobiology. The cytoskeleton is a highly dynamic web composed of different molecules including actin, tubulin, and vimentin, whose role is dependent on the context and structures generated in each condition, but it also relies on the cell type. In this way, the cells have a specialized manner to respond to the environment, always trying to survive and adapt. The response of adherent or nonadherent cells involves different mechanisms, including surface molecules, like integrins and selectins that help with adhesion, and generate a link between the physical microenvironment from outside and within the cell. Thus, the cytoskeleton provides the support for stem cells in culture conditions or homing and establishment inside the body. Considering that, in both cases, the mechanisms are not completely understood, this section focuses solely on the physical signals the cells receive, excluding soluble factors, which are also relevant, as most of the literature already addresses them.

Cytoskeletal rearrangement is induced by several stimuli like chemokines, growth factors, differences in the stiffness of a substrate, and others. As mentioned above, the extracellular environment is connected to the inner cell, including the nucleus [7, 8]. In that case, the characteristics of the substrate may generate a reaction inside the cells, and the reaction is led by a reorganization of the F-actin structuring and the modulation of actin dynamics, like polymerization/depolymerization cycles.

In this context, actin reorganization is required during stem cell differentiation, as well as adhesion, cellular spreading, force distribution, stress fiber formation, and others, all of which are completely dependent on the cytoskeleton, a complex scaffold constituted of different kinds of filaments distributed throughout the cytoplasm. Thus, rigidity, structure, and support are not the only functions, but also subcellular organization, inner transport of molecules, motility and migration, cellular division, and mechanotransduction.[9–11]

4. Nano topography of Biomaterials and the Role of the Cytoskeleton during Stem CellInteraction

Actin and tubulin, in contrast to vimentin, are cytoskeletal molecules generating, in a highly dynamic fashion, specialized structures in just a few seconds after a signal is initiated. This property provides a cell the capability of adapting if the microenvironment changes, or if it is required in other processes,like cell migration.

Stem cells share exceptional plasticity and adaptation to microenvironmental conditions. According to the plasticity potential, cells respond and adhere easily,

acquiring a spherical or spread shape. Stem cells are unspecialized with a remarkable potential to renew themselves, as well as the potential to differentiate into mature specialized tissue; both functions are maintained in balance. With invitro models, these properties can be manipulated by employing different strategies, like cocktails ofgrowth factors. Another approach is to use scaffolds, like mechanical cues.[12,13] which can be manipulated to generate specific changes in cellular shape, spreading, rearrangement of F-actin, stress fiber formation, and inducing adhesion patterns and differentiation.[2] (Fig 2).

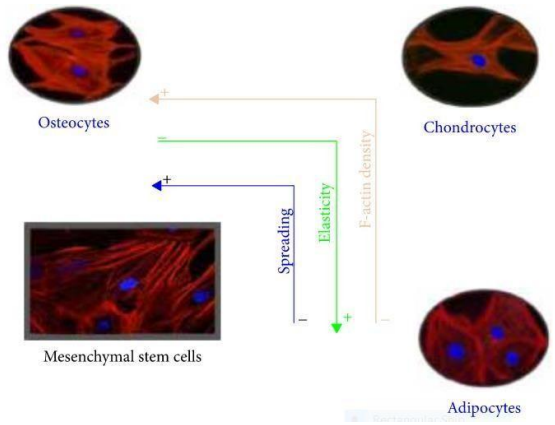

Fig. 2: Actin cytoskeleton in stem cell differentiation. Cellular spreading (blue line), elasticity (green line), and F-actin density (orange line) are some of the properties that change during stem cell differentiation and can predict the cell's fate. (By Dr. Ambriz, 2018).

In the human body, stem cells interact with ECM, which vary in composition depending on the site. ECM is mainly composed of collagen, fibronectin, and laminin as well as proteins like glycosaminoglycans and proteoglycans. Niche compositions rely on the abundance of these molecules and contribute to the differentiation process in which stem cells generate specific adhesionarrangements, maintaining or modifying its cellular shape and migration capability to other sites.

When stem cells are maintained in culture, it is crucial to avoid any possible stimulus that can trigger the differentiation process. For example, fibronectin binds to integrins like α5β1, α4β1, and αvβ3.

This interaction results in an intracellular actin reorganization and induces a specific change of shapeand the activation of intracellular signaling pathways, which results in chondrocyte differentiation. On the other hand, in vitro self-renewal of ES cells has been shown to be dependent on the interaction with type I or type IV collagen substrates.[2]

Induction of morphological changes of hMSCs has been studied by the generation of specific micropatterns.[39,40] causing topography-mediated differentiation.[40] ECM proteins have been used to "print" patterns, lines, or dots, with different spacing between each one, resulting in the induction of tension or compression, as the cells attempt to generate different distributions of FAs.

To illustrate the influence of nano topography, consider nanoislands of fibronectin. With spacing below 60 nm, it is possible to generate FAs, while longer spacing results in impairment of FA formation and cellular spreading. Moreover, the organization of patterns seems to be significant since disordered nanopatterns do not cause cellular spreading. Furthermore, FA maturation and cell adhesion strength were constant at 60 nm of spacing, in comparison to 70 and 120 nm of micropatterned substrates.[39] These results are highly significant if it is considered that biomaterials, with specific micro- or nanopatterned designs, can modulate the maturation of FAs, which in turn has consequences in stem cell proliferation, self-renewal, and differentiation, as described above.

5. Mechanical cues guide development

The growth, differentiation and morphogenesis of a developing embryo is dependent on intrinsic andextrinsic mechanical forces that drive the assembly of cells and promote growth into higher-order structures. Cell-cell adhesion transmits tensile forces, and, as embryonic development progresses, cells become mechanically coupled to matrix proteins in tissues by adhesion molecules (Fig. 3). This coupling helps to drive morphogenesis and maintain the position and fate of stem cells in their niche. This mechanical coupling enables storage of information over time. For example, changes in ECM induced by cells early in development can mechanically trigger changes in interacting cells at a later stage. Moreover, during morphogenesis, mechanical cues can be propagated over long distances more rapidly than biochemical cues (for example, the transmission of forces in highly elastic substrates such as elastin is almost instantaneous).[3]

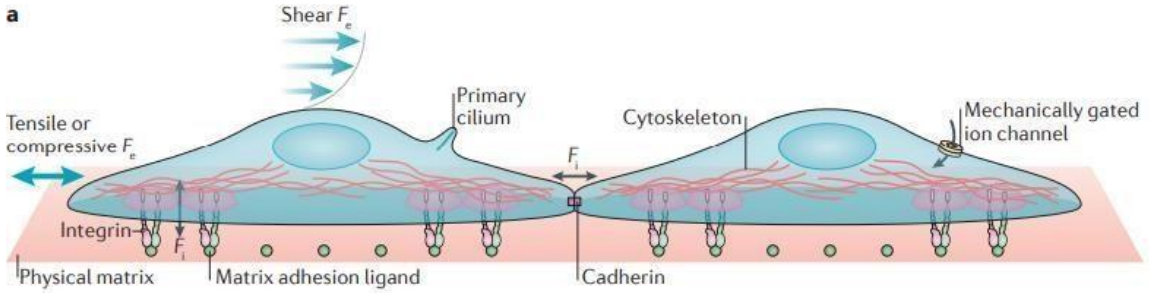

Fig. 3: a | Intrinsic, or cell-generated, forces (Fi) are generated intracellularly and transferred to other cells through cell–cell junctions, such as cadherin receptors, or via traction on extracellular matrix (ECM) adhesion ligands that are bound to integrin receptors. Cells are directly coupled by cell–cell junctions, which link the intrinsic forces of one cell to the cytoskeleton of another. Indirect mechanical coupling between cells occurs by intrinsic forces exerted on the ECM, to which two or more cells are adhered. Physical properties, for example, the elastic modulus, E, of the ECM, govern how mechanical cues are transduced. Extrinsic forces (Fe) are externally applied by shear or tensionand/or compression on cells, and they can be sensed by mechanically gated ion channels, changes inreceptor–ligand binding, deformation of the cytoskeleton and the primary cilium. The cytoskeleton generates and transfers forces from membrane proteins to intracellular structures, such as the nucleus.

6. Generation of a functional organ from a single adult tissue stem cell

To demonstrate whether there are true stem cells in a given tissue, one needs to show that a single stem cell purified from the tissue has the capability of generating the entire organ. Up to present, it has been elegantly shown by independent groups that the mammary gland and the prostate can be generated in vivo from a single adult tissue stem cell.[14-16] Two laboratories have reported independently that single stem cells isolated from adult mouse mammary glands are able to produce secretory mammary glands. when they are transplanted in the fat-pad in mice.[14,15] It is long believed that there are stem cells in the mammary glands because this organ has the capability of undergoing extensive growth at puberty and a second phase of expansion and retraction during pregnancy under the regulation of estrogen.[17] However, due to the lack of defined markers, there has been no reliable method to isolate mammary stem cells. This hypothesis was not proven until thework by the two groups. Based on some previous work by Clark and colleagues,[18] Shackleton et al. isolated putative mouse mammary stem cells using specific cell-surface markers (Lin-, CD29hi, CD24+) by FACS. They demonstrated these LinCD29hiCD24+ mammary cells have in vitro sphere formation capability and the ability to repopulate all mammary epithelial cells after transplantation into the fat pad. Importantly, the investigators employed a lineage-tracer in the stem cells so to follow their ultimate phenotypes in vivo. They showed elegantly that a single cell within the LinCD29hiCD24+ population of the mouse mammary gland, marked with a LacZ report transgene, can reconstitute a completely functional mammary gland in vivo. Notably, the transplanted cell contributes to both the luminal and myoepithelial lineages and generates milk-producing lobuloalveolar units during pregnancy. This is the first report to demonstrate that a single adult tissue stem cell has a multi-lineage differentiation capacity to produce a functional organ in an in vivo setting.

7. Stem cell-derived hepatocytes

***Embryonic stem cell-derived hepatocytes* (*ESC-Heps*)**

ESCs are derived from the inner cell mass of the fertilized egg, which is pluripotent, and can be culturedindefinitely in an undifferentiated state and have the potential to differentiate into three germlayer cell types. They have been shown to give rise to functional hepatocytes that effectively integrate into and replace injured parenchyma in many devastating liver diseases. As one of the most important organs, liver participates in various physiological activities, including the production of bile and albumins, metabolism of toxins and drugs, and maintenance of glucose and lipid balance. Therefore, a severe hepatic injury can cause serious consequences despite the great regenerative capacity of a liver, particularly for patients who have inherited metabolic disorders or chronic liver diseases such as liver cirrhosis. Basically, transplantation of a living donor liver is a gold standard for treatment of severe liver failure. However, the shortage of suitable donors constitutes a major obstacle for prompt treatment. Meanwhile, immune rejection is another potential issue after liver transplantation, which requires long- term immunosuppressive management.[6] Definitive endoderm (DE) cells are the precursors of both the liver and pancreas, and they have to be induced to undergo hepatic and pancreatic differentiation (Murry and Keller, 2008).

Moreover, the expression of alpha fetal protein (AFP), albumin (ALB), and a biliary molecular marker appear sequentially, suggesting the differentiation of ESCs recapitulates the normal developmental processes of the liver. There are some issues with these the current differentiation protocols, including spontaneous differentiation, low yield, the presence of undefined and xenogenetic compounds, necessity of cell sorting for specific cell lineages, considerable enzymatic stress during repeated culture (Haque et al., 2011) and cellularheterogeneity in the culture.[5]

The signalling molecules bone morphogenic protein (BMP) and FGF that have been implicated in hepatic differentiation during normal embryonic development and have been shown to play pivotal roles in generating hepatic cells from DE cells derived from ESCs. Using a set of human adult markers, including CAAT/enhancer binding protein (C/EBPalpha), hepatocyte nuclear factor 4/7 ratio (HNF4alpha1/HNF4alpha7), CYP7A1, CYP3A4 and constitutive androstane receptor, and fetal markers, including AFP, CYP3A7, and glutathione S-transferase P1, by 21 days of differentiation, ESC-Heps have the characteristics of fetal hepatocytes at less than 20 weeks of gestation, but extending the differentiation to 4 weeks does not improve cell maturation (Funakoshi et al., 2011). Li et al. (Li et al., 2011a) established an efficient method for the induction of mouse ESC-derived DE cells in suspension embryonic body culture.

The chemical activation of the canonical Wnt signalling pathway synergized with the activin A- mediated nodal signalling pathway to promote the induction of DE cells, and inhibition of BMP4 signalling by Noggin and activin A further improved the efficiency of DE cell differentiation. A combined treatment with Wnt3a and BMP4 efficiently differentiated human ESCs (Kim et al., 2013); after co-culture with STO feeder cells, human ESCs were able to differentiate into HLCs and cholangiocyte-like cells (Zhao et al., 2009). Forkhead box A2 (Liu et al., 2013) and synthesized basement membrane components (Shiraki et al., 2011) significantly increased the hepatic differentiation of ESCs.

Most studies demonstrating hepatic differentiation from ESCs have been based on embryoid body (EB) formation, aggregated colony formation in static culture. It has been shown that dynamic three- dimensional perfusion culture is superior to other culture systems for inducing maturation of ESCs into fetal hepatocytes and prolonging the maintenance of the hepatic functions of those cells. Ten pathways that were significantly upregulated in cells differentiated in a bioreactor compared to cells grown in static culture were shown to be highly related to liver functions (Sivertsson et al., 2013). The differentiated phenotype was sustained for more than 2 weeks in the three-dimensional spheroid culture system, which is significantly longer than in monolayer culture (Subramanian et al., 2014). EB-derived cells grown in a rotating bioreactor exhibited higher levels of liver-specific functions thanthose in static culture (Zhang et al., 2013a). The hollow fiber/organoid culture method allows for cultured ESCs to form an organoid, and the differentiating ESCs reach a level of functionality comparable to or better than that of primary mouse hepatocytes (Amimoto et al., 2011). Differentiated cells grown on a biodegradable polymer scaffold and a rotating bioreactor also exhibit morphologic traits and biomarkers characteristic of liver cells (Wang et al., 2012). Significantly upregulated hepatic gene expression was observed in the hepatic differentiation hollow fiber-based three- dimensional perfusion bioreactors with integral oxygenation culture group (Miki et al., 2011).However, most of the current three-dimensional differentiation configurations involve interruptive operations during the multistaged differentiation process, which might impose unwanted influence on cellular differentiation. Off-the-shelf micro-stencil arrays were developed to generate adherent multilayered colonies composed of human ESC-derived cells; the microscaled multilayered colonieswith uniform and defined sizes constrained within the microwells are composed of more homogenousand mature HLCs with significantly lowered AFP expression and elevated hepatic functions.[5]

8. Engineering a whole organ via decellularization of matrix bio scaffolds and recellularization with stem cells

Organ formation requires not only stem cells but also the surrounding stem cell niche or microenvironment and extracellular matrix participation. In fact, three-dimensional tissue scaffold has been demonstrated to be very critical for organ regeneration. Decellularization and recellularization of matrix bio scaffolds have been shown to be a promising approach for whole-organtissue engineering in recent years.[19] Donor organs such as the heart,[20] liver,[21] lung,[22] kidney,[23] and bladder,[24] can be decellularized to an acellular biologic scaffold material and then be recellularized with functional parenchymal cells and/or selected progenitor cell populations. Encouraging results have emerged in animal model studies.

Taylor and colleagues have generated a bioartificial heart which has a heart structure with appropriate cell composition and cardiac pump function using this attractive technique in 2008.[20] They perfused heart with 1% SDS, a strong ionic detergent, to produce acellular scaffolds, preserved extracellular matrix and original microvascular network, and then repopulated these heart-like constructs with neonatal cardiomyocytes and aortic endothelial cells. When proper physiological stimuli were given, the recellularized organ in the culture was able to further mature and display beating behaviors. Rhythmic contractions were observed by day 4, pump function generated by day 8 and cardiac architecture was formed, as confirmed by histological analysis. The bioartificial heart pumped with an amazing function, which was equivalent to about 2% heart power of an adult or 25%heart function of a 16-week fetus.

Uygun and colleagues modified Taylor's perfusion decellularization technique to achieve transplantable liver grafts in 2010.[21] The ischemic livers were perfused through the portal vein with SDS. The intact lobular structure and vascular network were retained in the decellularized liver bioscaffold. The reseeded adult hepatocytes and nonparenchymal cells were able to repopulate to create a recellularized whole liver graft. The graft was transplanted as auxiliary heterotopic graft with portal vein arterializations and animal was shown to be

viable for 8 hours after transplantation. The hepatocytes in the recellularized liver graft were metabolically active with functions of urea and albumin secretion. Similarly, Niklason and colleagues regenerated lung grafts using the decellularization and recellularization strategy in 2010.[22] They removed cellular components from lung matrix, preserved hierarchical branching structures of the airways and vasculature bed in the extracellular scaffold, and reseeded pulmonary and vascular endothelial cells in the acellular lung matrix. The engineered lungs presented pneumonocyte viability and function after implantation, as evidenced by oxygen exchange.

These exciting studies on complex organs, such as the heart, liver and lung as described above, provide insights into the power of the methodology. The technology has multiple advantages. First, a bioartificial organ could theoretically solve the problem of lifelong immunosuppression after organ transplantation. Second, the preserved extracellular matrix and three-dimensional scaffold provide important signals for engraftment of repopulated cells, survival of engrafted cells, and function of newborn organ. Third, the vascular bed in the decellularized bioscaffold allows oxygen and nutrient rapid to be delivered after recellularization and reconnection to the circulation. The most important concern in the technology is the cell type and the source of cells used for filling the decellularized organ matrix. Different types of cells are needed to reconstruct the parenchyma, vasculature, and support structures underneath. An ideal strategy is that a stem or progenitor cell can self-renew and differentiate into heterogeneous types of cells as needed to form a functional organ. Currently, cell sources are broadly categorized. Stem or progenitor cells used for recellularization include embryonic stem cells (ESCs), fetal cells, adult-derived stem or progenitor cells and adult tissue-derived inducible pluripotent stem cells (iPSCs).

Non-stem or progenitor cells used for repopulation are usually parenchymal cells (e.g. cardiomyocytes, hepatocytes, pneumonocytes), vascular cells (e.g.endothelial cells) and supportive cells (e.g. fibroblasts). Whether autologous or allogeneic cells are utilized will depend on cell numbers required and if the need is immediate. In short, decellularization and recellularization technique is a potential breakthrough in whole-organ tissue engineering.[4]

9. Stem cell therapy

Bioartificial liver (BAL) as a temporary liver supporting device can provide essential hepatic functions for the patients who are waiting for the transplantation of living donor livers. Conventional BALs work by the extracorporeal circulation of patients' blood through the devices, which have some limitations such as large and complex equipment. Therefore, there are urgent needs to enhance the hepatic functions of BALs while simplifying and minimizing their overall complexity and size, respectively. Cell therapy is a novel approach for the treatment of liver diseases by direct injection offunctional cells. Hepatocyte is the most important component of liver, which consists of ~60% of the total hepatic cell population and is responsible for the majority of liver functions. To retain at least 30% of the minimal liver functions, approximately 8.4×10^{10} hepatocytes are required, which posesa huge challenge for the treatment of liver failure only using autogenous cells, due to their few numbers of normal hepatocytes in vivo and limited proliferation capacity in vitro, not to mention theneed for other non-parenchymal functional cells (liver sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, and cholangiocytes). Furthermore, low cell survival rate and poor cell engraftment are the common issues during infusion of single-cell suspensions in a conventional cell therapy. Therefore, seeking an alternative cell source and enhancing cell delivery efficiency are two potential solutions for the effective treatment of liver failure.

Typically, there are two major roles of stem cells and progenitors in liver regeneration, including paracrine effect and hepatic differentiation. The former targets host cells for activation and acceleration of self-healing, while the latter produces new hepatic tissues to replace the injured liver.

Paracrine effect

The secreted growth factors involved in paracrine effects affect numerous intra- and extra-cellular signaling pathways, which may trigger a series of reactions promoting liver regeneration. For example, small hepatocyte-like progenitor cell (SHPC) clusters, one type of cells that have some phenotypes similar to hepatocytes, that appeared in injured rat livers induced by administration of retrorsine (Ret) and 70% partial hepatectomy (PH) increased in number and size after implantation of Thy1+ cells, one type of hepatic progenitor cells. The liver regeneration was enhanced through the IL17RB signaling pathway regulated by Thy1+ cell released extracellular vesicles (EVs). Yu et al. showed that hypoxia-preconditioning of bone marrow-derived mesenchymal stem/stromal cells (BMSCs) promoted liver regeneration after the infusion of cells into the portal vein of a rat model with 85% hepatectomy due to their enhanced expression of vascular endothelial growth factor (VEGF). Liver regeneration was also enhanced in a partially hepatectomized mouse model following infusion of conditioned medium of liver-derived MSCs through promoting cell proliferation and reducing proinflammation. Such therapeutic efficacy could be further promoted by using conditioned medium from adipose-derived mesenchymal/stromal cells (ASCs) in a hypoxic condition (1% oxygen) via the JAK/STAT3 signaling pathway. In another study by Lee et al. Liver regeneration in the partially hepatectomized mice was observed after intravenous administration of the exosomes derived from lipopolysaccharide-preconditioned ASCs. The superior therapeutic efficacy of MSCs and their exosomes for early hepatic ischemia-

reperfusion injury (IRI) was reported by Anger et al. The secreted paracrine factors such as VEGF and hepatocyte growth factor (HGF) from the EPCs could promote hepatocyte proliferation and angiogenesis of resident liver sinusoidal endothelial cells (LSECs) in addition to the induction of apoptosis in hepatic stellate cells (HSCs). However, it is notable that such paracrine effect can also aggravate the cirrhosis if circulating EPCs are derived fromabnormal donors which might be due to the distinct functions of the two cell subpopulations, early EPCs and outgrowth EPCs that are involved in inflammation and angiogenesis, respectively.

Growth factors are demonstrated to be the functional components of exosomes, therefore their targeted and sustained delivery may further promote liver regeneration. For example, Yu et al. Recently reported that cell proliferation of hepatocytes was markedly promoted after implantation of VEGF-loaded nanofibers into rats with 70% hepatectomy. Sustainable release of HGF, one type of paracrine growth factors, from carboxymethyl hexanoyl chitosan (CHC) hydrogel was also reported to enhance cell proliferation of iPSC-derived hepatocyte-like cells and maintain their hepatic functions in vitro. Meanwhile, the reduced area of hepatic necrosis and increased survival rate of thioacetamide (TAA)-induced liver injured mice were evidenced after the administration of HGF in vivo. Moreover, multi-dose administration of insulin-like growth factor-1 (IGF-1), another exosome component, was also recently reported to reduce collagen deposition in mice by Fiore et al., indicating the amelioration of liver fibrosis.[6]

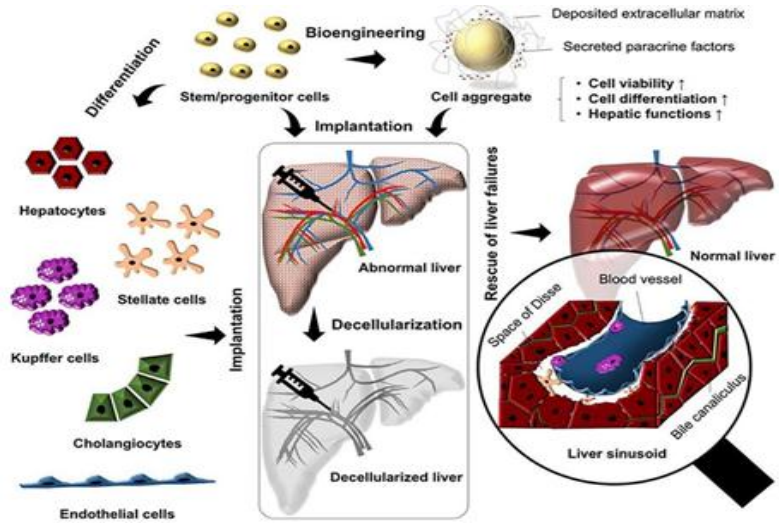

Fig. 4: A schematic illustrates the use of stem cell therapy, cell aggregate-based or decellularized liver scaffold-based tissue engineering strategies for the rescue of liver failure. To reconstruct a normal liver, stem cells/differentiated cells/bioengineered cell aggregates can be implanted into the abnormal liver or its decellularized counterpart.

10. Bioengineering of vascularized hepatocyte aggregates

Owing to the importance of vasculatures for normal physiological liver functions, various strategies have been employed to guarantee the supply of oxygen and nutrients to hepatic cells. One strategy is to implant hepatocyte aggregates near the existing blood vessels in vivo to promote angiogenesis.[25] The direct co-culture of hepatocytes and endothelial cells is another strategy for bioengineering of vascularized liver constructs.[26,27] The increased hypoxia-inducible factors at the center of hepatocyte aggregates can function as the chemoattractant, which results in the invasion of endothelial cells and the formation of capillaries. However, the fusion of these cell aggregates should be minimized to avoid the central necrosis of large aggregates and maintain the high surface-to- volume ratio for the effective exchange of nutrients and gases. To achieve these goals, Pang et al.23 used fiber fragments as the spacers to separate vascularized hepatocyte aggregates from each other and improve the supply of nutrients and oxygen. In a follow-up study, they further fabricated a Nylon 12-based 3D perfusion system composed of 43 chambers to house hepatocyte/endothelial cell aggregates, which could be theoretically scaled up to a clinically-relevant size (500cm3 in volume) (Figure 4). Both cell viability and hepatic functions were improved during the perfusion culture.[28] Moreover, they also seeded hepatocyte and endothelial cell hybrids inside the customized micro-scale scaffolds. The pores and intersecting hollow channels of the scaffolds allowed sufficientpenetration of the medium throughout the scaffolds when packed in a bioreactor for perfusion culture, leading to high cell viability and enhanced hepatic functions.[6]

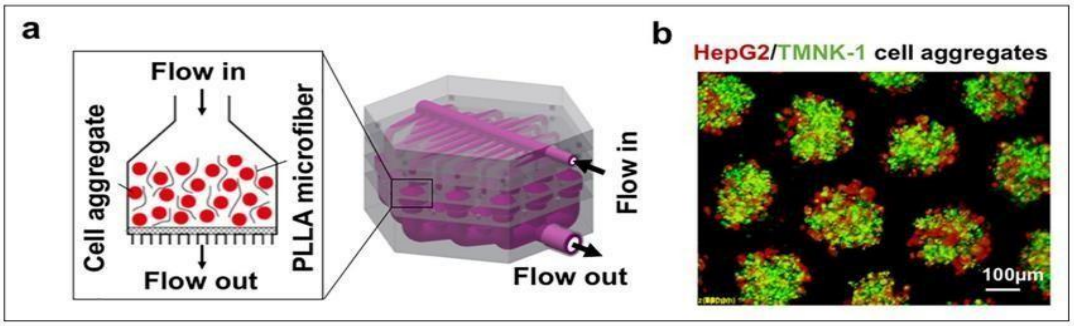

Fig. 5: Bioengineering of a cell aggregate-based bioartificial liver: (a) dynamic culture of hepatocyte (HepG2)/endothelial cell (TMNK-1) aggregates in a customized 3D perfusion system and (b) Immunofluorescent staining of HepG2/TMNK-1 cell aggregates in the microwells 24h after cell incubation. Red: HepG2 cells; green: TMNK-1 endothelial cells. Scale bar: 100μm. Adapted with permission from Pang et al.[28]

11. Cancer Stem Cells: Targeting Their Biomechanics

The complexity within stem cell niches elicits the interaction of CSCs with other cells. All of them could be involved in some kind of regulation but these aspects need to be defined specifically, as they are all related with those molecules involved in biophysical interactions of CSCs. Inside of cancer cell niches, several types of cells exist, such as tumor, stromal, and vascular cells where as part of them, CSCs are maintained in a quiescent stage until adequate conditions, including cellular and molecular events, induce them to proliferate, invade, and metastasize.[29]

CSCs have clear properties of self-renewal, clonal tumor initiation capacity, clonal long-term repopulation potential, transitions from a nonstem cell state to stem cell state, evasion of cell death, metastasis, and dormancy for long periods of time.[30]

CSCs can also be activated by direct or indirect interactions with different cell types present inside of niches and by biophysical interactionswithin cancer cell niches surrounding ECM or ECM molecules.[31,32] In those niches, the local cells produce factors capable of stimulating CSCs, inducing angiogenesis, and recruiting immune and other stromal cells that secrete additional factors while promoting tumor cell metastasis and invasion. CSCscan also produce exosomes, which facilitate ingress of RNA molecules that facilitate the ingress of multidrug resistance in tumor cells.[30]

In tumor niches or tissues, there is a production and concentration of several molecules that could activate CSCs. Biochemical and biophysical signals come from growth factors, cytokines, and matrix- remodelling proteins.[29] Potentially, all of them are capable of activating and/or inducing growth and differentiation of quiescent CSCs that develop into more aggressive stages. Part of those molecules are noncellular components derived from degradation of ECM due to the action of matrix metalloproteinases coming from activated cells inside of niches; the importance of the produced ECM is due to its constitution remaining as a physical barrier, as well as the integrity of CSCs blocking any possible harmful condition such as the action of chemotherapeutic agents found in solid tumors. Therefore, the release of cytokines, growth factors, and other molecules enable the degradation of the ECM because of the action of metalloproteinases which are factors facilitating angiogenesis, tumor cell metastasis, and invasion associated with therapeutic resistance. Cross talk between CSCs and their niches has the basis of activation of these types of cells.[30]

It is clear that those factors present in the microenvironment of cells have an important influence on different stem cell phenotypes. Such factors are composed of materials surrounding the cells that can compete with biochemical supplements. They influence or induce the activation and/or the differentiation of stem cells by inducing or activating signalling pathways through mechanotransduction because of its mechanosensing.[32] Therefore, those conditions involving cancer therapy, where there is a manipulation of the components of the microenvironment of cancer niches or tissues, could produce an effective strategy for cancer treatment or the induction of resistance to cancer therapy and the prevention or the maintaining of malignancy and metastasis of CSCs.[30]

Materials in cellular environments are capable of being inductors/activators of stem cells which is a crucial consideration for CSC niches. If these molecules are part of the surrounding niches, their surfaces could become inductors of CSC activation that can be involved in mechanotransduction and mechanosensing. These conditions could trigger growth, expansion, and drug resistance as shown by these types of cancer cells. It is mandatory to consider whether their engineering and utilization be the reason why some quiescent CSCs become capable of expanding and developing resistance to anticancer drugs.[33] There are several properties of synthetic materials which can induce changes in cellular activity, and these include stiffness, molecular flexibility, nanotopography, cell adhesiveness, binding affinity, chemical functionality, degradability, and/or degradation by-products.Materials could produce specific stem cell behavior which need to be always considered during the design and use of materials,[32] and CSC biology and

niche factors have to be involved during the use of materials for regenerative medicine purposes.

The significance of cell fate and mechanotransduction has been discussed above, but to understand how they are involved in CSCs' progression to metastasis requires describing the signalling pathways activated when a mechanical disruption occurs and cell-cell contact is lost. There is a tensional homeostasis within cells when there is a disruption, and it may play a role in oncogenic transformation. Considering that stiffness is measured by mechanosensors in cancer stem cells and solid tumors have high mechanical stress, this may impede drug delivery driving tumor progression. Biomechanic forces can drive tumor aggression in the case of a mesenchymal-like switch, developingtumor-initiating or stem-like cell properties, as well as elevated tissue mechanics promoting aggression. These events open the possibility of manipulating mechanical properties of CSCs to breakdrug resistance by its stemcell phenotype.[34]

12. Technology platforms for cancer cell mechanobiology studies

There has been a growing body of work linking cellular biomechanics with a variety of platforms, which have been engineered to perform mechanical measurements and have generated robust datasets in different cell types (Table 1). The most common explored method is mechanical probes, represented by Atomic Force Microscopy (AFM) and Magnetic Tweezer (MT).[35] In cell biology, typical force ranges from 1-10 pN in the case of kinesin and myosin, ~1-200 pN for protein-protein interactions, ~100 pN for partial protein unfolding, and ~1 nN to 10 pN for migrating or contracting cells[36] Mechanical properties of living cells can be probed and characterized by recording force-displacement curves under 1 pN-1 µN force on a part of a cell surface. AFM is a powerful tool for imaging biological samples with sub-nanometer resolution, providing tremendous insight into cell surface features and cell mechanical properties, as well as cellular processes that affect the mechanical properties of living cells.[37] The main parts of the AFM are the cantilever, the tip that is mounted to a soft cantilever spring, the sample stage, and the optical deflection system, which consists of a laser diode and a photodetector. Force spectroscopy relies on measuring the force as the tip is pushed toward the cell, indented into the sample, and subsequently retracted. A force-displacement curve canbe obtained by monitoring the deflection of the cantilever; this data enables extraction of a value for the Young's modulus, *E*, of living cells.

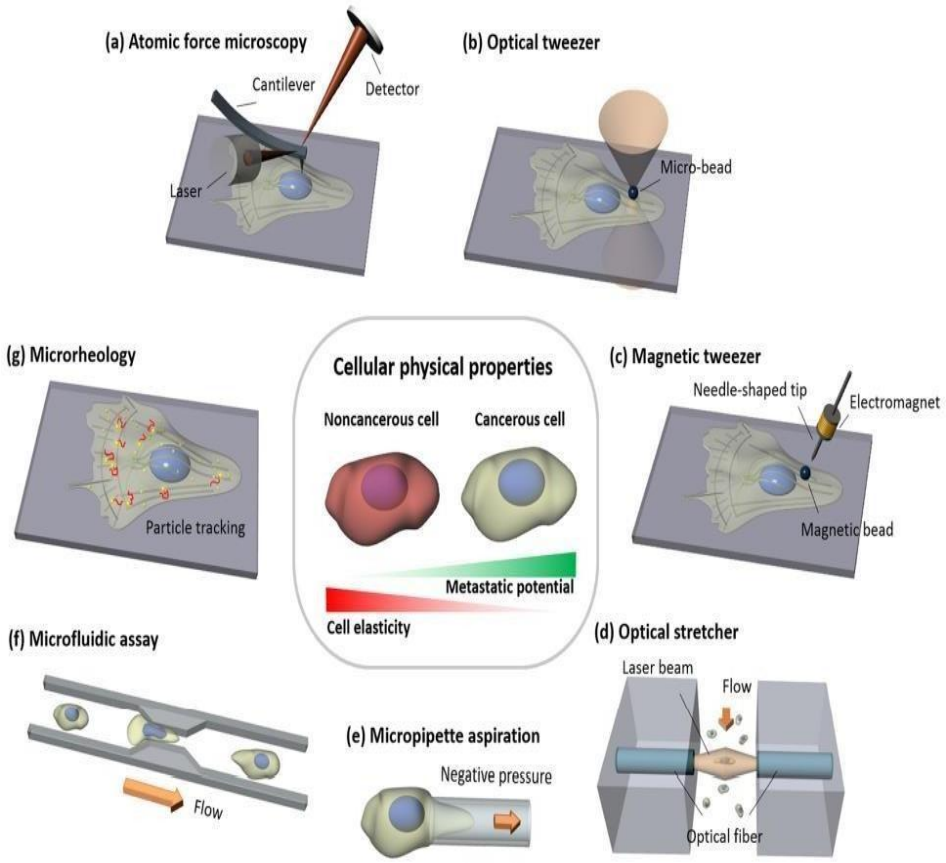

Fig. 6: Representative experimental tools for measuring physical properties of the cell. (a) Atomic microscopy, (b) optical tweezer, (c) magnetic tweezer, (d) optical stretcher, (e) micropipette aspiration, (f) microfluidic assay, (g) microrheology.[38]

Table 1: Cellular biomechanical studies with different technology.

Tissue type	Cell line / Clinical sample	Technique
Breast	Benign (MCF-10A) vs. cancerous (MCF-7) human breastepithelial cells	AFM
	Benign (MCF-10A), non-invasive malignant (MCF-7), and highly-invasive malignant (MDA-MB-231) breast cancer cells	AFM
	Benign (MCF-10A) vs. non-metastatic tumor breast cells (MCF-7)	Microfluidics
Higher deformability in invasive cells (transformed MCF7) than nonmetastatic MCF10 and non-transformed MCF7 breast cell		Optical deformability
	Suspected metastatic breast cancer cells	AFM
	Normal breast and breast cancer tissues	Spectrumreponse
	T47D and MCF7 breast cancer cells, fresh tissue samples	AFM
	Breast tissue samples	Indenter
Bladder Urothelial	Normal (Hu609, HCV29) and bladder cancer cells (Hu456, T24,BC3726)	AFM
	Normal human urothelial (SV-HUC-1) and bladdercancer cells (MGH-U1)	AFM
	Non-malignant urothelial cell HCV29 and transitional bladdercancer cell T24	

13. Cancer Mechanosensation

The cancer microenvironment can enhance or inhibit cancer cell behaviors such as sensitivity of cellular microenvironment, motility, polarization, cytoskeletal organization, and proliferation via a mechanical signal transmitting process termed mechanotransduction. To decipher these mechanotransduction pathways, various bioengineering platforms that manipulate cellular microenvironments have been developed. Four major approaches ECM stiffness control, micropatterned cell confinement, micro/nano topographic substrate, and ECM specificity are introduced as representative methods for microenvironmental mechanosensing of cancer cells.[38]

13.1 ECM Stiffness. Cancer cells can actively recognize the physics of the extracellular microenvironment through the mechanosensory proteins and integrin adhesion network.89 With these mechanosensory proteins that connect ECM to the cytoskeleton, signal cascades are involved in interpreting extracellular mechanical signals to biochemical signals. To discover the interaction between cancer cells and ECM stiffness, various substrates with diverse compliant materials instead of the traditional tissue culture flask or rigid glass have been used.

Polyacrylamide (PA) hydrogel is extensively used to mimic mechanical rigidity of the extracellular microenvironment of in vivo conditions because it is easily synthesized and the range of stiffness values could cover various soft tissues in the body.

Various studies have observed cancer cell behaviorusing PA gel with a wide range of substrate stiffnesses. Schrader et al. have shown that increasing substrate stiffness (1−12 kPa) can promote proliferation of hepatocellular carcinoma cells (Hun7, HepG2) via the mechanotransduction pathwayinvolving protein kinase B (PKB/Akt) and phosphorylation of STAT 3 (signal transducer and activator of transcription 3). Furthermore, chemotherapy-induced apoptosis of hepatocellular carcinoma cells is decreased on a stiff substrate due to reduction of the poly-ADP-ribose polymerase cleavage. Nukuda et al. have also reported thata stiff substrate (126 kPa) can enhance the expressionof MMP7 compared to a soft substrate (2 kPa).They found that YAP, epidermal growth factor receptor (EGFR), α2β1 integrin, and myosin regulatory light chain (MRLC) increased MMP7 expression in T84 colorectal cancer cells. McGrail et al. have demonstrated that the cell adherent index, traction forces, cell spreading, and polarizationof the phosphorylated myosin light chain (pMLC) of ovarian cancer cells (SKOV3, OVCAR3) are increased on a soft substrate (2.83 kPa) compared to those on ahard substrate (34.88 kPa).Mesenchymal phenotypes are more dominant on a soft substrate than on astiff substrate, indicating that the malignancy of ovarian cancer cells is increased on a soft matrix. However, other groups haveshown opposite results. McKenzie et al. have demonstrated that cell spreading, focal adhesion (vinculin, focal adhesion kinase phosphorylation) formation, traction forces, and myosin light chain phosphorylation of epithelial ovarian cancer cells (SKOV3) are increased with increasing substrate stiffness (3− 125 kPa). They also demonstrated that hard stiffness could increase YAP translocation into the nucleus and metastasis of cancer cells. Cell stiffness is also an important index to identify thestate of cells, and cellular modulus can also be changed in response to substrate modulus. Indeed with increasing substrate stiffness, the cellular stiffness of normal hepatic cells (L02) and hepatocellular carcinoma cells (HCCLM3) is increased.

Collagen, one of the primary constituents in mammalian ECM, is conveniently used to prepare rigidity-tunable in vitro cell culture matrixes. Increased secretion of collagen around a tumor is known to promote invasion and metastasis of cancer cells. Intracellular compliance of prostate cancer in a 3D collagen matrix is increased with increasing collagen matrix (stiffness range: 0.16 to 8.73 Pa). Matrix ligand density increases with increasing collagen matrix stiffness since the pore size of the collagen matrix is correlated with matrix mechanics and

ligand mediated cell−matrix interaction.

Matrix stiffness plays an important role in various activities of cancer cells, including progression, proliferation, attachment, mesenchymal phenotype change, migration, and invasion. Cancer cells on soft substrates have more a dynamic interaction with the surrounding microenvironment to search forexternal cues for migration, whereas cancer cells on a stiff substrate are relatively ready to be activated for malignant transformation by up-regulating MMP, β1 integrin, and focal adhesion kinase(FAK).[38]

13.2. Micropatterned Cancer Cell Confinement. With recent advances in microtechnology, robustmethods have been developed to control the cellular microenvironment to characterize cellular properties. One representative method that has been widely used to confine cells in a specific microenvironment is micropatterning of cell adhesive proteins or nonadhesive materials on a substrate. The microcontact printing (μCP) technique allows a substrate to be functionalized with awide range of adhesive proteins at a size of nanometers. Fibronectin is one of the most widely used proteins in μCP experiments because it is an extracellular cell adhesive protein that plays a major rolein cell motilities such as initial cell attachment, growth, migration, and wound healing process. Moreover, understanding the directional motility and bias motion of cancer cells is important since they are relevant to physiological processes of metastasis. The Grzybowski group has fabricated micropatterned ratchets to facilitate directional cell migration.

Interestingly, in a specificallydesigned micropattern, where linear ratchets with spikes inclined at 45° (short base of a unittrapezium), two types of cancer cells (B16 cell and MDA-MB-231) moved in opposite directions.Cell−cell communication between cancer cells and stromal cells or between cancer cells and othertissue cells plays an important role in cancer development. To speculate how stromal cells affectcancer progression, more than two types of cells can be cultured together. A coculture system with aprecisely controlled microenvironment is often used to understand how cancer cells affect propertiesof noncancerous cells, and vice versa, affecting them either directly through physical/chemicalcell−cell interactions or indirectly through soluble cytokines. To mimic these coculture systems, cellmicropatterning using the μ-eraser technique or stencil micropatterning technique has beendeveloped. Zhong et al. have cultured lung cancer cells (A549) on poly(lactide-coglycolide) (PLGA)and then removed A549 cells in specific regions by pressing cell layers with a PDMS stamp. Afterusing the μ-eraser technique, osteoblast cells (hFOB 1.19) were seeded to investigate the effect ofcoculture on two cell types. Such a method isalso used for drug evaluation since bone is one of themain organs metastasized by lung cancer.

The efficacy of doxorubicin was decreased, whereasalkaline phosphatase expression of osteoblast cells was elevated in the coculture system.

13.3. Micro/Nano Topographic Substrate. Topographic features of cancer cell microenvironment can also change cell motility and cancer progression. For instance, cancer cells can recognize the collagen fibers and migrate along with the same direction of collagen fiber. Generally, the ECM recognition, mechanotransduction, or migration pathway of cancer cells is not different from that of normal cells, i.e., noncancerous cells. However, cancer cells have higher elasticity than normal cells and thus are highly invasive, as mentioned earlier. Migration and invasion of cancer cells are complicated processes. They are affected by a combination of external signals. To explore the motility mechanism underlying cancer invasion, recent studies have determined how topographical stimulations could affect cancer behavior by culturing cancer cells on a micro/nano topographic substrate. Fibroblastic sarcoma cells (SaI/N) placed on dense microcircular posts or a microgrooved pattern showed increased persistence and migration speed compared to those lying on a flat substrate, indicating topographic variation guided cells to migrate less randomly. Comparisons of noncancerous breast epithelial cells (MCF-10A) and breast cancer cells (MDA-MB-231, MCF-7) have also been performed under the topographic cues. While the proliferation of cancer cells was not changed regardless of substrate topography, that of noncancerous cells was significantly decreased when cells were cultured on 2−4 μm microgrooves or micropillar substrates. In the same manner, cancer cells were resistant to Rho-ROCK-Myosin signaling activated by topographic cues. They consequently continued uncontrolled proliferation.

13.4. ECM Specificity. The extracellular matrix (ECM) is a collection of extracellular macromolecules secreted by various types of cells and acts as a key component of tissue. It provides the physical and biochemical support of an organ. ECM has a wide range of mechanical and various biochemical properties. ECM can change shape, polarization, proliferation, apoptosis, motility, and development of cancer cells via mechanotransduction signals of cell ECM. ECM also plays a role asa blocking barrier to prevent drug diffusion to cancer cells, causing chemoresistance or inhibiting cancer cell migration and invasion to neighboring tissues.

Laminin-332 (also known as laminin-5), an ECM component with α3Aβ3γ2 chain composition, is secreted by cancer and stromal cells and plays a critical role in epithelial cell adhesion to the basement membrane. Some studies have reported that expression of laminin-332 is increased in several types of cells such as gastric carcinoma, oral squamous cell carcinoma, and hepatocellular carcinoma cells.

Laminin-332 binds to the β4 subunit of integrin and promotes migration, invasion, and proliferation of tumor cells. Furthermore, transforming growth factor-β and laminin-332 are involved in epithelial- mesenchymal transition (EMT). In the cancer−stromal interface, laminin-332 and membrane type 1- MMP are overexpressed, consequently promoting tumor invasion by remodeling the microenvironment.

Fibronectin plays an important role in cell adhesion by binding integrin at the cell membrane. Fibronectin-binding cells exert force through the integrin family, such as α3β1, α4β1, α5β1, αVβ1, αVβ3, and αVβ6. A wide range of cell types can bind to fibronectin with these receptors, consequently affecting various cancer cell behaviors. Among these integrins, α5β1 integrin is markedly active in malignant cancerous cells. Generation of fibronectin fibril is essential to physiological and pathological processes such as actin− myosin contraction, cell adhesion, development, invasion, and differentiation. Park and Schwarzbauer have found that exogenous fibronectin can promote EMT by up-regulating cadherin, vimentin, Snail, and MMP2. They also reported that fibronectin-induced EMT was dependent on Src kinase, ERK/MAP kinase, and transforming growth factor (TGFβ).

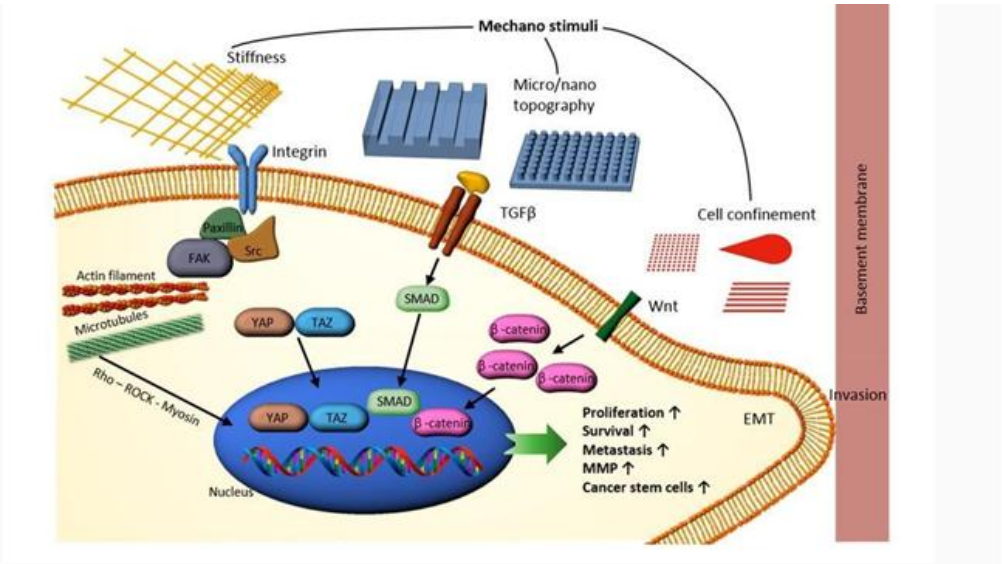

Fig. 7: Molecular mechanism of cancer cell mechanosensation. Cancer cells respond to mechano stimuli such as ECM stiffness, micro/nano topography, and cell confinement. Mechanical signals are transmitted to focal adhesions such as paxillin, FAK, and Src through integrin that activate small GTPase Rho, ROCK, and myosin in series. In addition, mechanical signals such as matrix rigidity induce activation of TGFβ and Wnt/βcatenin. These activations lead to nuclear translocation of transcription factors such as YAP, TAZ, SMAD, and β-catenin. Mechanosensation of cancer cells promotes cell proliferation, survival, and MMP secretion that are required for cancer metastasis.

14. Metastatic Cancer

Metastatic dissemination, spreading of cancer cells from a primary or initial site to surrounding or distant sites, is a unique characteristic of cancer cells, which typically involves a series of sequential processes of EMT, invasion, intravasation, and extravasation (Fig 8).

In order to initiate and complete the metastatic sequential, cancer cells must change their shape to an invasive phenotype (EMT), invade the surrounding cells or ECM (invasion), penetrate the circulatory system (intravasation), and exit from the blood or lymphatic microvessel (extravasation). Cancer metastasishas been studied for more than one century. One famous hypothesis is “seed and soil” proposed by Stephen Paget in 1889. It means that cancer metastasis depends on cross-talk between the cancer andthe specific microenvironment of the organ. This hypothesis has been widely accepted and provides a guide to cancer and metastasis research.

14.1 EMT

EMT can alter cellular properties from epithelial phenotype, high adhesiveness, and low migratory ability to mesenchymal-like phenotype with neoplastic properties, low adhesiveness via loss of E- cadherin, and increased invasiveness. Mechanical stimuli generated by cellular microenvironment stiffness can affect the regulation of EMT by TGFβ, a potential EMT inducer. In addition, EMT can be regulated by mechanotransduction pathways through transcription factors, such as twist-related protein1 (TWIST1). Nuclear translocation of TWIST1 is promoted by releasing TWIST1- binding proteins, such as Ras GTPase-activating protein binding protein 2 (G3BP2), in a stiff matrix. Many proteins are known to characterize EMT such as E-cadherin, vimentin, YAP, and transcriptional coactivator TAZ. A loss of Ecadherin, increase of vimentin, and nuclear localization of YAP have been observed in the EMT process. Elevation of specific ECMs including laminin-332, periostin, andfibronectin has been observed in EMT

as mentioned above. Although these phenomena with 3D in vitro studies provide robust mechanistic insights to EMT of cancer cells, promising results are not simply derived or matched with in vivo models.

14.2 Cancer cell invasion

To penetrate the circulatory system (intravasation process), cancer cells start to migrate and breach an extracellular basement membrane (BM), a sheet-like specialized ECM that acts as natural barrier of the primary tumor. The BM is composed of reticular connective tissue and basal lamina containing multiple molecules of type IV collagen, laminin, entactin/nodigen, and proteoglycans. An increase of tumor stiffness with type I collagen and fibronectin enrichment can lead to cancer cell proliferation and invasion. Invasive cells feature invadopodia, distinct actin-based dynamic protrusions that can degrade the ECM component by recruiting MMPs.These extended membrane protrusions are enriched by an invasion-related complex such as cortactin, paxillin, or protein kinase C. Cancer cell invasion is enhanced in response to mechanical stimulation with increased invadopodia. Applying transient tugging forces to collagen and the fibronectin substrate can down-regulate β1 integrin, Rac1- GTP, PAK1 activation, and LIM kinase 1 activation and up-regulate cofilin activation with longer and matured invadopodia. A recent study has reported that colorectal carcinoma cell spheres can disseminate to peritoneum without an EMT process. They showed that the tumor sphere can maintain epithelial organization to enrich E-cadherin and β-catenin between cancer cells. In addition, the tumorsphere is polarized to the outward of part of the sphere that can be observed by apical markers including ezrin, villin, atypical protein kinase C, CD133, phospho-ezrin-radixin-moesin, and Na+/H+ exchanger regulatory factor. Furthermore, the tumor sphere can maintain its spheroidal shape and collectively migrate into collagen gel or matrigel. Whether EMT is an essential process for invasion and metastasis or not is not clear, and the correlation between EMT and metastasis will be an excitingarea for future research.

14.3 Intravasation

Following the metastatic cascade of the EMT process and local invasion, cancer cells will enter into the blood or lymphatic circulatory system (intravasation) for spreading nearby or at distant sites. Intravasation leads to circulating tumor cells (CTCs) in the circulatory system. These CTCs presenta high risk for secondary metastasis. Cancer cell intravasation depends on penetration through connective tissue and the cell−cell junction between endothelial cells. In this process, the shape of the cytoplasm and nucleus can be severely deformed unless endothelial cells and the cell junction are retaine. In vitro systems including the transwell invasion assay and artificial microvessel system using a microfluidic device have been developed to elucidate or visualize cancer intravasation. The Kamm group has developed a microfluidic chip-based platform to mimic the tumor-vascular interface in 3D. Invasion into the endothelial barrier of fibrosarcoma cells (HT1080) was visualized in the presence of tumor necrosis factor alpha.

For in vivo intravasation studies, two methods are commonly utilized. One is quantitative measurement of intravasation by analyzing circulating tumor cells, while the other is obtaining intravital imaging of cancer cell invasion through the blood vessel wall. The Segall group has reportedthat overexpression of the epidermal growth factor receptor in adenocarcinoma cells (MTLn3) can enhance intravasation and metastasis to the lung.188 Direct imaging of cancer cell intravasation wasconducted using multiphoton microscopy. Advanced in vitro microenvironments with increased complexity and in vivo intravital imaging will promote understanding of the interaction between cancer cells and stroma during metastasis.

14.4 Extravasation

For complete metastasis, cancer cells in blood or the lymphatic circulatory system (called CTCs) haveto adhere to the capillary and exit to form metastasized tumors at a secondary site. The first step for extravasation of cancer cells surviving from shear stress in the circulatory system is attachment to endothelial cells. Endothelial cell adhesion is then needed to stabilize cancer cells that will exit the circulatory system by squeezing the cytoplasm and nucleus. A number of extravasation and intravasation studies have been performed using in vitro and in vivo 3D modeling. Extravasation canbe classified as paracellular extravasation and transcellular extravasation based on the route of cancer cell extravasation. Paracellular extravasation is related to adhesion molecules (including E-selectin, P-selectin glycoprotein ligand 1, CD24, CD44, mucin 1, and galectin-3-bining protein) and chemokines and their receptors including CXC-chemokine ligand 12, CXC chemokine receptor type 4 (CXCR4), and CXCR7.194 Transcellular extravasation was recently observed in an in vitro study using colorectal cancer cells and leukocytes. With this mechanism, cancer cells can exit the circulatory system and penetrate through endothelial cells, not between endothelial cells. However, further studies will determine whether this extravasation exists for other types of cancer cells or in vivo.[38]

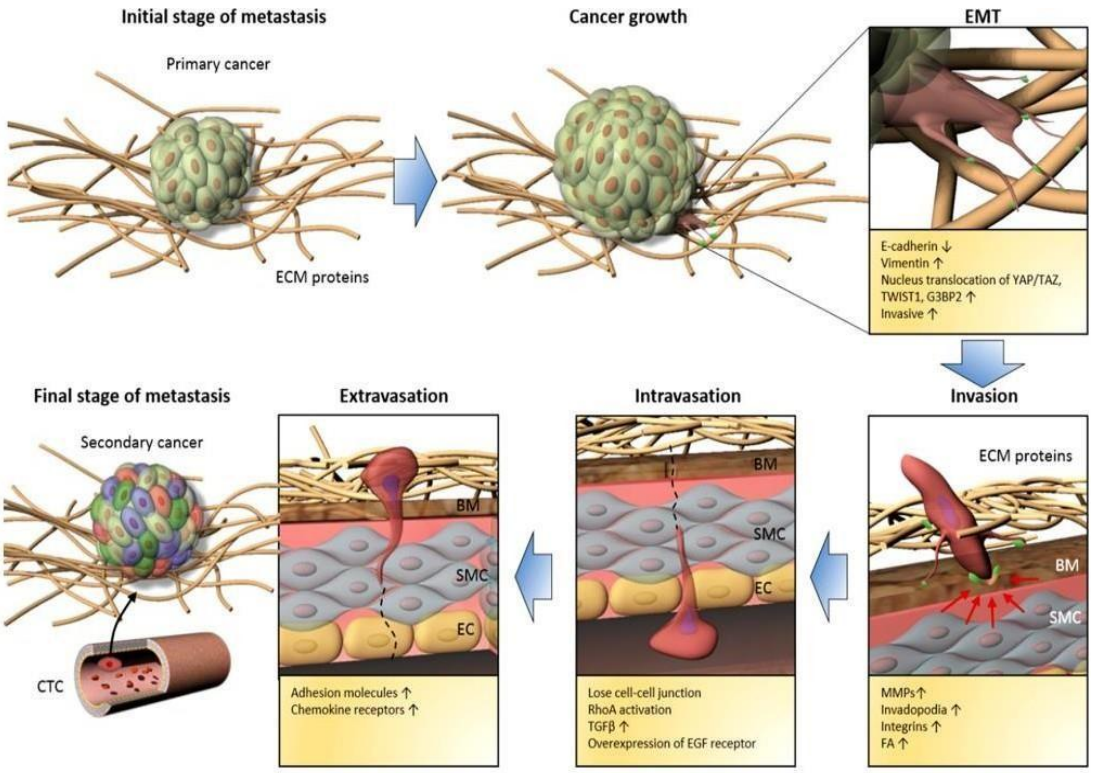

Fig. 8: Schematic of cancer metastasis. Aggregation of primary cancer cells grows out by the ECM proteins at the initial stage of metastasis, when some cancerous cells follow epithelial to mesenchymal transition with EMT-associated features, including a loss of E-cadherin and an increase of N cadherin, vinculin, and nucleus translocation of YAP/TAZ, TWIST1, and G3BP2. Before penetrating the circulatory system, the cancer cell breaches the basement membrane (BM) at the invasion stage. In this stage, MMP recruitment, invadopodia, integrins, and FAs are activated. Moving to distal sites, cancer cells start to penetrate the circulatory system with intravasation features such as a loose cell−cell junction, RhoA activation, increased TGFβ, and overexpression of EGF receptors. Once a cancer cell penetrates into the circulatory system, CTC floats with the bloodstream and then exits from the circulatory system to form a metastasized tumor at a secondary site in response to environmental cues.[38]

CONCLUSIONS

The role of mechanical forces in the induction of differentiation of stem cells. While the details associated with individual studies are complex and typically associated with the stem cell type studied and model system adopted, certain key themes emerge. First, the differentiation process affects the mechanical properties of the cells and of specific subcellular components such as the nucleus. Secondly, that stem cells are able to detect and respond to alterations in the stiffness of their surrounding microenvironment via induction of lineage-specific differentiation. Finally, the application of external mechanical forces to stem cells, transduced through a variety of mechanisms, can initiate and drive differentiation processes. Understanding cytoskeleton arrangement and its implication during stem cell interactions with biomaterials, as well as the importance of Nano topography, creates new aspects of integration with other fields of knowledge, in order to improve cell-based therapies and regenerative medicine. It is a big risk to design and build a biomaterial without evaluating their physical aspects, such as the stiffness, porosity, nano topography,chemical composition, and interaction with other types of surfaces. Moreover, the aberrant growth ofcells or the wrong differentiation of the stem cells can be triggered by alterations in their physical microenvironment.

As indicated, under these considerations, activated cells could be redirected to produce pathological situations or in the case of CSCs to develop resistance to anticancer drugs due to epigenetic changes. It is important to carry out the characterization of the new biomaterials, in order to establish if they are adequate for the terms of any potential use in regenerative medicine A large body of work has shown that tissues and cells respond to mechanical cues in their environment,and it is increasingly apparent that tumor cell invasion at least to some extent is mediated by biomechanical factors. In fact, recent evidence has suggested that in some cases mechanical signalingoverrides genomic defects andelicits a normal phenotype in cells that otherwise would have been transformed (Gieni and Hendzel,in press). There are now a wide range of in vitro biomechanical testsystems that may be used with cancer cells, and the challenge will be to develop practical in vivo model systems of tissue and cell mechanobiology.

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