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A Review of the Effects of Extracellular Matrix Stiffness On Cellular Activity

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ABSTRACT

The extracellular matrix is a vital component of a cellular system. It is responsible for several metabolic processes within cells and its properties can have major effects on cellular activity. Recent research has begun to understand the role of physical interactions between the matrix and the cell on cellular growth and repair. In this work, we review the role of the stiffness of the extracellular matrix in determining cellular activity. We first review the effect on stem cell differentiation through the influence of cytoskeletal feedback loops and induced traction stresses. We then discuss the effects of metabolic reprogramming in tumor progression via molecular upregulation of YAP/TAZ and genetic expression. Finally, we highlight the effects of the stiffness gradient dictated by durotaxis and induced hypoxia on the rate of tumor progression. We hope this review sheds light on the major impact that the extracellular matrix has on various biological activities.

Introduction

The extracellular matrix (ECM) is a connective tissue that plays a key role in tissue development. It maintains the physical elements and morphology of the muscle and the physiological functions in cellular control. The ECM is composed of multifunctional gel-like fibrous structures scattered throughout the tissue. It contains various proteins which interact with the cell to regulate processes along the lines of cellular activity. Many components of the ECM affect cellular behavior. For instance, the ECM can directly bind different types of cell surface receptors or co-receptors to mediate cell anchorage and pathways involved in intracellular signaling and mechanotransduction(Gattazzo et al., 2014). Moreover, it promotes organogenesis and development of a developing fetus(Urbancyzk et al., 2019). The most employed method to investigate such effects involves in vitro testing through cell culturation in different mediums. For example, Halliday and Tomasek(Halliday et al., 1995) conducted experiments in vitro to examine mechanical factors affecting fibronectin fibril assembly. In efforts to manipulate the cellular matrix and environment of culutration, the researchers used two collagen gels subject to different environments. A stabilized collagen gel was used to induce stress within the cell culture and a stress-free, relaxed gel was used as a control. Collagen was chosen because it is a key component in cell scaffolding. Alternate manipulations of in vitro conditions can be seen through Beta 1 integrin binding to regulate cellular traction(Gershlak et al., 2015); using photocleavable hydrogels to fabricate the network's structure under cytompatable conditions(Kloxin et al., 2009) or using 3D matrices to recapitulate key elements of a tumor microenvironment(Gu et al., 2015).

The focus of this investigation is to understand the impact that varying stiffness of the Extracellular matrix has on cell activity. The internal environment of the ECM is very complex - involving a multitude of proteins both fibrous and glycosaminoglycan. The cross-linking of these compounds and their quantities heavily contribute to the manipulation of the stiffness of the cell(Huang et al., 2021). The paper will review other published materials that have cited impacts of stiffness as manipulated in vitro on stem cell differentiation, metabolic reprogramming and tumor progression.

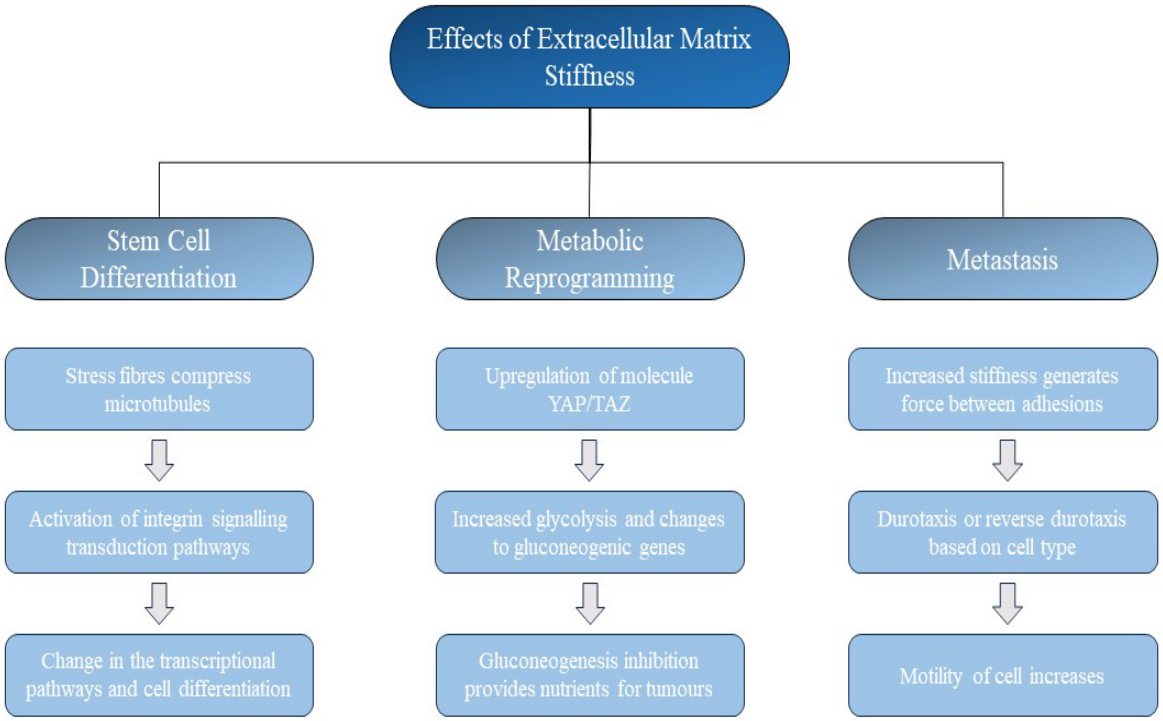

Figure 1. A summary of the effects of extracellular matrix stiffness on cellular activity

Cell-Cell Matrix Interactions

The impact of mechanical forces on a cellular level has been a subject of increasing interest(Wells, 2008) Every cell and its matrix interact through the formation of multi-protein adhesion structures, focal adhesions, fibrillar adhesions and podosomes that link the ECM to the cytoskeleton of the cell. ECM components are linked to each other through diverse protein and carbohydrate-binding domains ("What are cell-matrix adhesions?," n.d).

Within the body there exists a wide range of cellular stiffnesses, from brain to skeletal muscle tissues. Each region of the body creates a microenvironment, or a niche, to allow for physical manipulation of the cell strain and type for optimal functioning. Aside from growth, stiffness of the extracellular matrix has an impact on the enzyme-catalyzed reactions or on the progression of tumor cells.

Effect on Stem Cell Differentiation

Stem cells are unspecialized cells that possess the ability of self-renewal and potency allowing for differentiation into several cell types. Stem cells are classified based on their ability to divide and their source. Adult stem cells are very specific to their source, requiring a particular niche constructed by cell-specific cues. These cues are incredibly complex but broadly categorized into three types: cell-cell, cell-matrix, and cell-biochemical factor. Understanding the various cell interactions is crucial in fields of research development and therapeutic applications.

A key factor influencing stem cell behavior is stiffness. The impact of stiffness on stem cell differentiation can be linked to the initial traction stress. The initial traction stress is balanced out by the microtubules

resisting compression forces. The cell senses this through the activation of integrin-mediated signal transduction pathways. Through cytoskeleton-based feedback loops, a cell changes its maximal mechanosensitivity close to the microtubule compression determined by matrix stiffness, ultimately directing cell differentiation(Lv et al., 2015). The effect of matrix stiffness has also been investigated in vitro. For instance, a study was conducted to identify the optimal elastic modulus in a hydrogel for neural stem/progenitor cell (NSPC) differentiation and proliferation (Leipzig et al., 2009). An NSPC cell was chosen because adult stem cells in the Central Nervous System(CNS) can propagate and differentiate into the primary cell types of the CNS. Native brain tissue is a soft tissue with a lower elastic modulus compared to skeletal tissue. The study found a correlation between the preference of cell culture and growth and stiffness of the surface. For example, neurons prefer compliant hydrogel surfaces whereas astrocytes prefer slightly stiffer hydrogel surfaces. Thus, when resting on a very soft substrate, hippocampus-derived NSPC differentiate into neurons, but form astrocytes on stiffer surfaces.

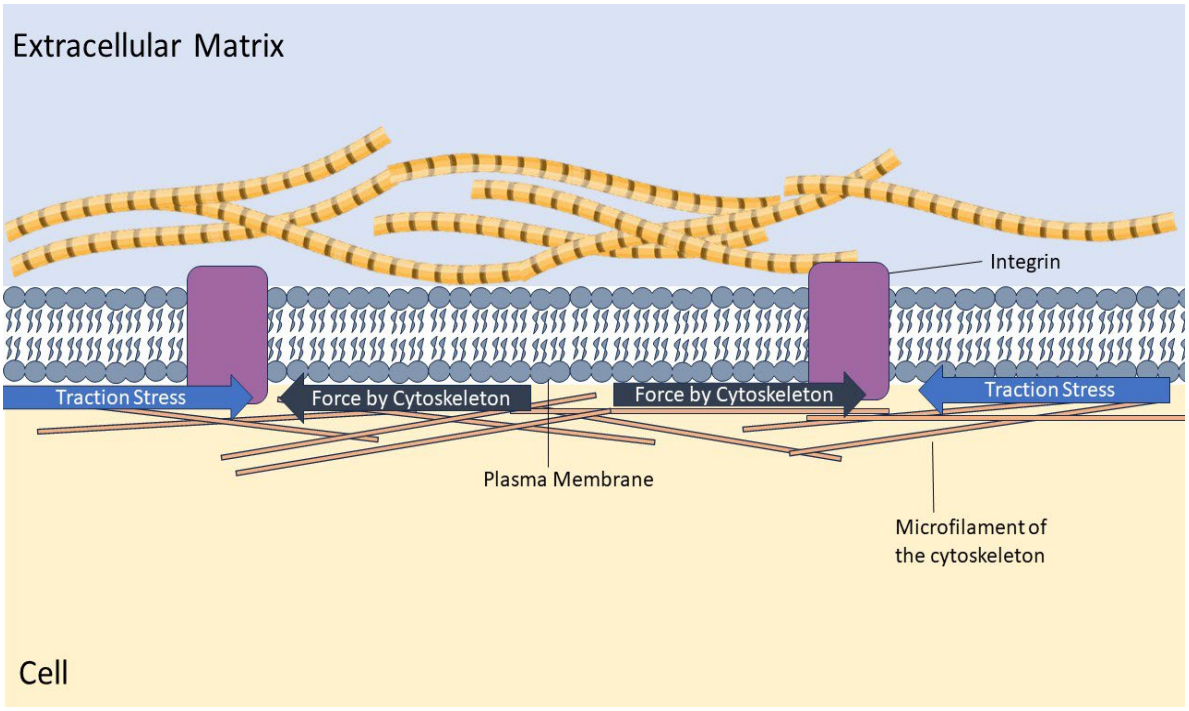

Figure 2. Stem cell differentiation by traction stresses and cytoskeleton feedback loops

Effect on Cellular Metabolism and Reprogramming

Metabolism is the collective referencing of the many enzyme-catalyzed reactions that take place in the body. It serves as a source of energy and biomass for cellular activity by regulating protein, lipid and carbohydrate expenditure(Ge et al., 2021). Cellular reprogramming refers to an activity by cancer cells during tumor formation whereby an increased uptake of energy is used to promote uncontrolled proliferation. Cancer cells can only achieve proliferation when granted access to sufficient energy for DNA, RNA, complex carbohydrates, proteins and lipids for mitosis. The ECM also can regulate cellular metabolism and reprogramming. For instance, inhibition of tumor growth and proliferation in in vivo and in vitro cultures can be manipulated through variable ECM stiffness(Ge et al., 2021).

Emerging evidence indicates that metabolism change occurs downstream of mechanotransduction signaling pathways that are activated by mechanical stimuli. A key molecule involved in the manipulation of glucose metabolism is YAP/TAZ. The molecule plays a key role in embryo and tumor development through

cell proliferation and survival. Through the upregulation of YAP/TAZ, glucose metabolism takes place. The upregulation is impacted by the stiffness of the ECM through various mechanisms, one of which is through influencing receptors.

Firstly, YAP/TAZ affects glucose metabolism through increased glucose transport protein expressions, which are regulated by cellular receptors. This is a direct result of increased genetic transcriptions promoting glucose uptake. For instance, it has been shown that the stiffer the extracellular matrix, the increase in YAP promoting aerobic glycolysis(Liu et al., 2020).

Secondly, increased stiffness of the matrix is linked to subsequent expressions of various enzymes involved in glycolysis. These enzymes include several kinases, lactate dehydrogenase, and fructose-2,6-biphosphatase. This was shown in a study on the migration of hepatocellular carcinoma cells(HCC), whereby the absence of YAP/TAZ in cultured HCC cell lines resulted in the downregulation of related enzymes. Another study done in pulmonary hypertension revealed that ECM stiffening resulted in sustained cell growth and migration and cascade signaling(Bertero et al., 2016).

Thirdly, is the impact YAP/TAZ has on gluconeogenic genes and their expressions.YAP inhibits the transcriptional coactivator PGC1α's ability to bind to and activate transcription from the promoters of its gluconeogenic targets, and the effects of YAP are blunted upon its knockdown. In vivo, constitutively active YAP lowers plasma glucose levels and increases liver size(Hu et al., 2017).

Both processes of promoting glycolysis and inhibiting gluconeogenesis support the growth of the cancer cell, which has an increased demand for nutrients. These processes allow for a greater supply of glucose to the tissue but result in nutrient starvation of surrounding healthy tissue and other structures.

Effect on Tumor Progression

The key element of cancer that makes it so deadly is its uncontrolled growth and ability to move and spread across the body. The spreading of tumors is particularly dangerous because when cancer grows it impairs the functions of surrounding organs. Unsurprisingly, in the progression of cancer cells, the microenvironment of the cell, especially its stiffness, affects the growth and movement of the cell.

Cell proliferation refers to the process by which a cancer cell replicates its genetic information and manages to create a duplicate cell. As another form of cell growth, proliferation is dependent on the availability of nutrients and their supply to the cell. The impact of ECM stiffness is reflected through induced tension on the cytoskeleton. Cytoskeleton tension can inhibit or activate multiple signaling pathways of growth factors. Moreover, it can block or facilitate mitogenic activity in response to stimulations by ECM physical factors(Deng et al., 2022). The stiffness of the extracellular matrix impacts the motility of cells. For cancer cells, this would directly impact the rate of metastasis. Cells have been shown to show preferential directional motility, tending to migrate along 2D substrates from softer to stiffer environments in a process termed durotaxis(Zaman et al., 2006). Research suggests that as tumor microenvironments get progressively stiffer from cell growth, results in the activation of specific channels and increased malignancy(DuChez et al., 2019). Cancer cells, during metastasis, find easier movement through durotaxis. In special cases, the phenomenon of 'reverse durotaxis' was observed as well. The dual characteristic among cell movements allows for adaptability for cancerous cells for increased ability to move as dependent on stiffness gradients.

A secondary impact research involves the condition of Hypoxia. Hypoxia is a biological condition that refers to an oxygen-deficient state that the body is subjected to. The formation of a primary cancer tumor results in regions on tissues that receive a lower supply of oxygen as a result of increased distance from a blood vessel, and therefore, requires a greater diffusion pathway for oxygen(Gilkez et al., 2014). Hypoxia has been shown to stimulate the epithelial-mesenchymal transition(EMT). An EMT is a biological process that allows a polarized epithelial cell, to undergo multiple biochemical changes to form a mesenchymal cell phenotype, this results in enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production

of ECM components(Kalluri et al., 2009). Studies show that increased alignment of ECM might more readily establish a chemoattractive gradient that potentiates hypoxic signaling. Ready alignment of ECM is a function of stiffness, where increased stiffness suggests larger alignment. Increased ease of motility allows for a greater rate of metastasis.

Conclusion

In this work, we have reviewed the effects of the ECM on various cellular activities. We have shown how the ECM can affect stem cell differentiation through cytoskeletal feedback loops and induced traction stresses. We have also reviewed the various ways in which metabolic reprogramming during tumor progression can be affected by the ECM. Finally, we discuss the effect of the ECM on rates of metastasis. While many recent advances have been made in understanding the role of the ECM, the author believes several areas of research warrant further investigation. For example, the effect of cellular reprogramming in the formation and movement of cancer cells as influenced by mechanical forces is still elusive and requires more experimental data.

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