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Mechanogenomic coupling of lung tissue stiffness, EMT and coronavirus pathogenicity

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A B S T R A C T

In this Current Opinion, we highlight the importance of the material properties of tissues and how alterations therein, which influence epithelial-to-mesenchymal transitions, represent an important layer of regulation in a number of diseases and potentially also play a critical role in host-pathogen interactions. In light of the current SARS-CoV-2 pandemic, we here highlight the possible role of lung tissue stiffening with ageing and how this might facilitate increased SARS-CoV-2 replication through matrix-stiffness dependent epithelial-to-mesenchymal transitions of the lung epithelium. This emphasizes the need for integrating material properties of tissues in drug discovery programs.

1. Introduction

The recent outbreak of COVID-19 and its impact on the ageing population has exemplified the importance of understanding ageing tissue microenvironments and how they facilitate viral infection and replication. SARS-CoV-2 viruses are positive-strand RNA viruses and use both, transcriptional and translational mechanisms to regulate their pathogenicity [1]. These viruses mainly infect the upper respiratory tract in human populations. While infection rates appear to be similar in all age groups, morbidity and fatality rates have been shown to be significantly higher in the ageing population [2]. This is even more pronounced in ageing individuals with underlying chronic medical conditions. Given the detrimental global impact of SARS-CoV-2, it is critical to understand the coupling between SARS-CoV-2 pathogenicity and its interplay with ageing so as to integrate such understanding into drug discovery and repurposing programs [3].

A number of studies taking a materials perspective have revealed that tissue mechanical properties are altered during the process of ageing [4]. For example, the crosstalk between stromal cells and the epithelium as well as the endothelium is altered and leads to changes in the mechanical properties of the stromal microenvironment, as evidenced in tumors [5]. Notably, alterations in matrix stiffness play an important role in the homeostasis of epithelial cells [6]. As cells and tissues age, fibroblasts, which are critical to maintaining the stromal microenvironmental composition, undergo a transition to senescent states. Ageing also results in altered functional properties of fibroblasts, i.e., in their dynamic regulation of matrix deposition and matrix remodeling properties. Such imbalances in the ageing tissue microenvironment result in increased extracellular matrix protein deposition and hence altered tissue stiffness properties. A number of studies have shown that increased matrix stiffness leads to the epithelial cells switching to mesenchymal phenotypes [7,8]. We hypothesize that the increased epithelial-to-mesenchymal transitions in the ageing lung tissue could provide a microenvironment that facilitates coronavirus replication and increases pathogenicity in aged populations.

In the following sections, we first discuss the mechanical properties of the lung tissue and its alterations with ageing. We then briefly review matrix-stiffness dependent epithelial-to mesenchymal transitions in the context of ageing. Next, we highlight recent single-cell experiments linking epithelial and mesenchymal cell states to modular gene expression programs. Finally, we discuss how the mechanical state of cells may intersect with viral replication and nuclear mechanotransduction. Collectively, our review highlights the importance of the mechanogenomic coupling of tissue microenvironmental properties for disease initiation and progression, which we here discuss in the context of the SARS-CoV-2 outbreak.

2. Lung tissue stiffness and ageing:

The anatomy of the lung consists of a tracheal tube followed by the bronchial and the alveolar sacks. The lining of the lung is built out of ciliated epithelial cells coated with a mucosal layer. The epithelial cells

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are attached to the basement membrane connecting to the stromal microenvironment [9]. This microenvironment comprises of a number of cell types including fibroblasts. As cells and tissues age, fibroblasts undergo transitions to senescent states, leading to dysfunctional properties [10]. In particular, ageing fibroblasts exhibit increased matrix deposition, leading to alterations in the stiffness of the stromal microenvironment. In line with this, a number of recent studies have shown that the Young's modulus of the ageing lung tissue increases [11]. These experiments used force spectroscopy measurements applied onto lung tissue biopsies at subcellular, cellular and tissue scale to quantitatively establish the changes in the elastic modulus during the ageing process of the lung tissue. Such elasticity experiments combined with more recent single-cell sequencing experiments have highlighted the heterogeneous cell subpopulations present in lung tissues and how their gene expression programs are altered with ageing [12]. Importantly, the resulting alterations in matrix stiffness have been shown to be critical for epithelial cell homeostasis. Since coronaviruses infect the upper respiratory tract, it is therefore important to assess if the changes that occur in the lung tissue microenvironment during ageing could facilitate the viral pathogenesis (see Fig. 1).

3. Stiffness induced EMT

Epithelial-to-mesenchymal transitions are fundamental to early developmental programs [13]. In addition, such cell state transitions have become equally important in a number of disease progression models including cancer [14]. Epithelial cells are characterized by a tight coupling between cell–cell junctions and cell matrix interactions. These cells are in general cuboidal and with apical-basal polarity, and show up-regulation of e-cadherin proteins. In contrast, mesenchymal cells are more spindle-shaped and are stabilized by cell matrix interactions with increased actomyosin contractile machinery. These cells upregulate relatively more matrix proteins, such as the focal adhesion proteins including integrins, to stabilize cell matrix interactions. While mesenchymal cells are more migratory, epithelial cells are more sedatory. A number of experiments have revealed that the transitions from an epithelial to a mesenchymal phenotype could be triggered by altering matrix mechanical properties [15]. Epithelial cells plated on hydrogels with increasing stiffness induced the transition of epithelial cells to a more mesenchymal phenotype thereby also upregulating their matrix dependent gene expression programs [7,8]. Such a transition in the epithelial cell state suggests that softer substrates favor e-cadherin dependent junctional homeostasis, whereas stiffer substrates favor integrin-dependent cell-matrix homeostasis. Importantly, a number of studies have shown that epithelial-to-mesenchymal transitions could be the precursors to tumor initiation and progression [16]. For example, in breast tumors, epithelial cells that transition to mesenchymal phenotypes have been shown to lead to increased metastatic potential. Collectively, these studies suggest that matrix stiffness properties within in the tissue microenvironment, which could potentially be altered by the stromal cells, can facilitate epithelial-to-mesenchymal transitions with important downstream functional consequences.

Fibroblasts play a major role in this context by tuning the matrix stiffness properties [17]. These cells under normal homeostasis secrete extracellular matrix proteins to enable the matrix formation, as well as secrete matrix-metallo-proteinases (MMPs) to degrade the extracellular matrix. This dynamic balance between matrix deposition and degradation sculpts the normal tissue microenvironment. As fibroblasts age, the dynamic balance between their matrix remodeling properties becomes mis-regulated, thereby leading to increased matrix deposition and reduced matrix remodeling, for example in lung tissues [18]. Such matrix stiffness alterations associated with ageing are a hallmark of tissue fibrosis [19]. Increased stiffness and fibrotic behavior can also occur through mis-regulated matrix remodeling properties during wound healing [20]. Since the basal membrane of the epithelial cells are stabilized by the underlying extracellular matrix environment, any alterations in the matrix mechanical properties also regulate epithelial cell behavior. During ageing or mis-regulated wound healing such alterations in the fibroblast matrix remodeling properties and the downstream stiffening of the matrix can induce epithelial-to-mesenchymal transitions *in vivo*. We conjecture that the altered epithelial tissue homeostasis, i.e. heterogeneous epithelial and mesenchymal phenotypes within the ageing lung epithelium, could provide an important niche for pathogen infections such as coronaviruses.

4. Single-cell mesenchymal and epithelial phenotype responses

A number of recent studies have shown that the mechanical state of the cell, as defined by either the cell–matrix and/or cell–cell interactions within the tissue microenvironment, regulates cytoskeletal architecture to facilitate specific nuclear mechanotransduction pathways, which in turn differentially regulate gene expression [21,22]. We recently identified the cytoskeletal control of nuclear and 3D chromatin organization as an additional and important regulator of gene expression programs [23–25] (See Fig. 2). In particular, we showed that fibroblast cells in stretched or stiff versus relaxed or soft mechanical states show different cytoskeletal architecture, nuclear deformability, chromatin modifications and 3D chromosome organization patterns, and in effect differ in their overall gene expression programs. In particular, stretched/stiff cells show more of a mesenchymal phenotype with upregulation of the serum response pathway and its downstream target genes including genes involved in cell-matrix interactions, whereas relaxed/soft cells show more of an epithelial phenotype with upregulation of the NF-κB pathway and its downstream target genes, including genes involved in cell*cell interactions [23–24]. Interestingly, we also showed that mechanical stimulus, such as compressive load, similar as could be experienced in ageing tissues, leads to differential gene expression depending on the mechanical state of cells [26]. In addition, when cells in different mechanical states are stimulated with the same cytokine signal such as TNF-α, cells in both mechanical states activate the NF-κB nuclear signaling, but yet show distinct activation of NF-κB target genes, which could be explained by the distinct genome organizations in the two mechanical states [27] (See Fig. 3). It is known that coronaviruses make use of host cell immune response pathways including NF-κB signaling

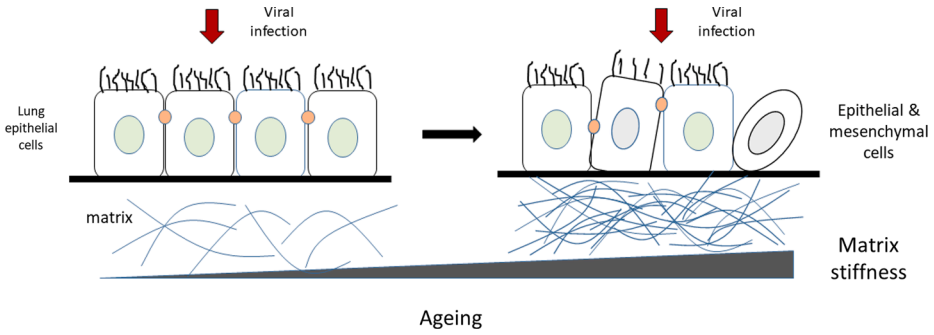

Fig. 1. A schematic of lung epithelial cells and the underlying matrix. The ageing tissue leads to increased matrix deposition, thus resulting in increased matrix stiffness. The schematic also depicts that such increased matrix stiffness results in the lung epithelial cells undergoing an epithelial-to-mesenchymal transition resulting in heterogeneous cell states with ageing. Our hypothesis is that coronavirus signals are differentially processed by the young and healthy epithelium versus the old and heterogeneous epithelium (shown in schematic).

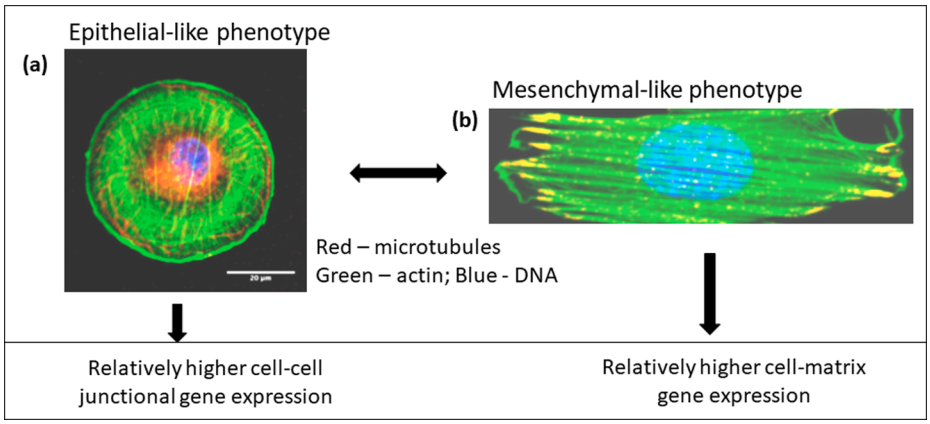

Fig. 2. Cells exhibit modular genetic programs depending on their mechanical states. Fibroblast cells plated on micropatterned substrates show that when subjected to relaxed geometric constraints the cytoskeletal organization shows an epithelial-like phenotype (a), while the same cells subjected to stretched geometric constraints exhibit a mesenchymal-like phenotype (b) with increased actin stress fibers and actomyosin contractility. The corresponding gene expression programs in the relaxed geometry show upregulation of cell–cell adhesion programs. In contrast, cells in stretched geometries exhibit upregulation of cell matrix programs. These experiments suggest that the gene expression programs are critically dependent on the cellular mechanical states. (Partially adapted from our paper in reference 23).

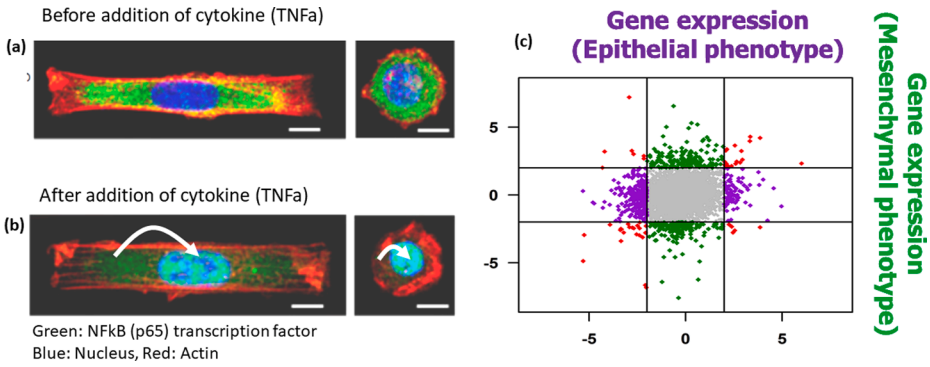

Fig. 3. Experiments to demonstrate the differential processing of cytokines depending on the mechanical states of cells. Stimulation through cytokines such as TNF-a activates downstream nuclear signaling of the NF-kB transcription factors. In (a) immunofluorescence experiments show the nuclear localization of p65 upon TNF-a stimulation in both stretched and relaxed cell geometries. As can be seen clearly in (b) the nuclear localization of p65 are similar in both geometries. However, the corresponding gene expression patterns either with cytokines or with compressive loading are very distinct depending on the mechanical state of cells (c). The relaxed epithelial-like phenotype differentially regulates a distinct group of genes when compared to the stretched mesenchymal-like phenotype. We suggest that such mechanical state dependent extra-cellular signal integration could explain the ageing-dependent coronavirus pathogenicity. (Partially adapted from our papers in references 26 & 27).

and we conjecture that the mechanical alterations in the aged lung epithelium could explain the age-dependent functional outcomes of SARS-CoV-2 infection.

5. Linking mechanical state of cells with SARS-CoV-2 replication and nuclear mechanotransduction:

Viruses have evolved to take advantage of the host cell signaling mechanisms to suppress inflammatory responses against their replication, as well as use host cell transcriptional and translational pathways to replicate themselves [1]. In particular, coronaviruses replicate completely within the cytoplasm, suggesting that their replicative mechanism may intersect with a host of cytoplasmic regulatory molecules. The recent SARS-CoV-2 cases have shown that while the infection rates are similar among different age groups, the virulence is higher in the ageing population [2]. This suggests that coronaviruses may intersect with ageing-dependent pathways to aid their replication. Since the first entry of coronaviruses in the upper respiratory tract occurs at the ciliated epithelium, it is critical to understand ageing-dependent alterations in epithelial tissue homeostasis. As we highlighted above, the epithelial layer in ageing lung tissues becomes stiffer and hence may induce epithelial-to-mesenchymal transitions, depending on the underlying matrix stiffness. Indeed, in recent tissue biopsies related to SARS-CoV-2 infections, one has observed highly infiltrated and fibrotic-like tissue remodeling [28]. These results may be indicative of the interplay between SARS-CoV-2 infection, its replication, and its downstream signaling impacts with ageing.

Host cells have developed a number of defense mechanisms through the inflammatory pathways such as the NF-κB signaling to signal to the immune cells for fighting the infection. In this context, we suggest that coronaviruses could alter such host-immune signaling in ageing populations driven by the alterations in the underlying tissue mechanical properties. As we described above, the mechanical state of cells and the resulting 3D genome organization is critical to regulate the NF-κB pathway upon stimulation with cytokines. Strikingly, by comparing the genes that were found to be differentially regulated by coronavirus infection in [29] with the genes that respond differentially to a cytokine in different mechanical states in [27], we found a high overlap including *EGR1*, *DDIT3*, *ATF3*, *IL6*, *CYR61*, *TNFAIP3*, *HSPA1A*, etc. [30]. This suggests that the epithelial phenotypes in younger populations and the heterogeneous epithelial-to-mesenchymal phenotypes in older populations could exhibit distinct downstream gene expression programs for the same viral signals. In particular, in aged populations the mesenchymal phenotype of cells infected with coronaviruses could enable dampening of inflammatory signaling while the more epithelial phenotype in younger individuals could trigger normal host-cell defense signaling mechanisms. Collectively, these results suggest that the material properties of the ageing lung tissue together with a more careful analysis of coronavirus replication pathways and their intersections with mechanotransduction pathways would be essential to shed light on the age-dependent pathogenicity of coronaviruses.

6. Conclusions: Opportunities for drug discovery

In this brief Current Opinion, we highlighted the importance of the mechanical properties of the tissue microenvironment as a potential key integrator of viral pathogenesis. This represents a novel dimension for exploring coronavirus infections and their replication in upper respiratory tract systems. The proposed interplay between tissue stiffness, ageing, and viral pathogenicity calls for an in-depth analysis of host-pathogen interactions taking into account the material properties of different tissues. We also suggest that the material properties of the tissue microenvironment and how lung cells within this microenvironment exhibit heterogeneous functional responses would be key to drug discovery programs. The marriage between material science and drug discovery in the context of major diseases is only in its beginnings and will be an essential step going forward for developing therapeutic interventions for the more recent viral diseases such as SARS-CoV-2.

In the context of ageing-dependent diseases, it would also be critical to combine material properties of tissues with single-cell transcriptomic data. This is particularly important since the ageing tissue exhibits more heterogeneous cell states. In this regard, single-cell transcriptomic data provides a window to exploring the coupling between single-cell mechanics, the tissue microenvironment, and functional response. Such datasets in the presence and absence of viral infections provide a direct snapshot of subsets of cells that could be poised for infections. Targeting these particular subsets of cells poised for infection and their microenvironment for therapeutic interventions would require a more mechanistic understanding of the ageing tissue and its cell mechanical heterogeneity.

Given the complexity of lung tissues, a major step forward in the current SARS-CoV-2 pandemic is to rapidly obtain post-mortem tissue biopsies for high-resolution correlative imaging from sub-cellular to cellular to tissue scale. Such imaging datasets could then enable fabrication of engineered tissue microenvironments for the analysis of coronavirus infections and for large-scale drug screening programs. Based on this idea, we recently developed causal network models integrating genomic, proteomic, and structural datasets within the framework of ageing and SARS-CoV-2 infection [31]. Such an analysis provided major insights to serine/threonine and tyrosine kinases as potential targets. These protein kinases are involved in signaling pathways that are activated by both, SARS-CoV-2 infection as well as matrix stiffness dependent cellular responses involved in ageing. Such integrative computational methods for drug discovery programs highlight the need for exploring materials perspectives to diseases. In this context, experimental programs would benefit largely by developing tissue organoid models that incorporate the appropriate *in vivo* tissue mechanical constraints. This would be highly valuable for large-scale drug screening for viral infections and as well as for various ageing-dependent diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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