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Evidence and therapeutic implications of biomechanically regulated immunosurveillance in cancer and other diseases

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Disease progression is usually accompanied by changes in the biochemical composition of cells and tissues and their biophysical properties. For instance, hallmarks of cancer include the stiffening of tissues caused by extracellular matrix remodelling and the softening of individual cancer cells. In this context, accumulating evidence has shown that immune cells sense and respond to mechanical signals from the environment. However, the mechanisms regulating these mechanical aspects of immune surveillance remain partially understood. The growing appreciation for the 'mechano-immunology' field has urged researchers to investigate how immune cells sense and respond to mechanical cues in various disease settings, paving the way for the development of novel engineering strategies that aim at mechanically modulating and potentiating immune cells for enhanced immunotherapies. Recent pioneer developments in this direction have laid the foundations for leveraging 'mechanical immunoengineering' strategies to treat various diseases. This Review first outlines the mechanical changes occurring during pathological progression in several diseases, including cancer, fibrosis and infection. We next highlight the mechanosensitive nature of immune cells and how mechanical forces govern the immune responses in different diseases. Finally, we discuss how targeting the biomechanical features of the disease milieu and immune cells is a promising strategy for manipulating therapeutic outcomes.

Disease progression is often associated with dynamic changes in mechanical properties at cellular and tissue levels. Such mechanical aspects of disease progression have been extensively studied in various pathological conditions, including cystic fibrosis[1], viral[2] and bacterial[3] infections, inflammation[4] and cancer[5]. Evidence shows that immune cells experience and respond to passive and active mechanical forces throughout their lifecycles. These mechanical forces have important roles in regulating the development, activation, migration, differentiation and effector functions of immune cells[6]. In particular, growing evidence has shown that T cells are mechanosensitive[7]. Upon cognate

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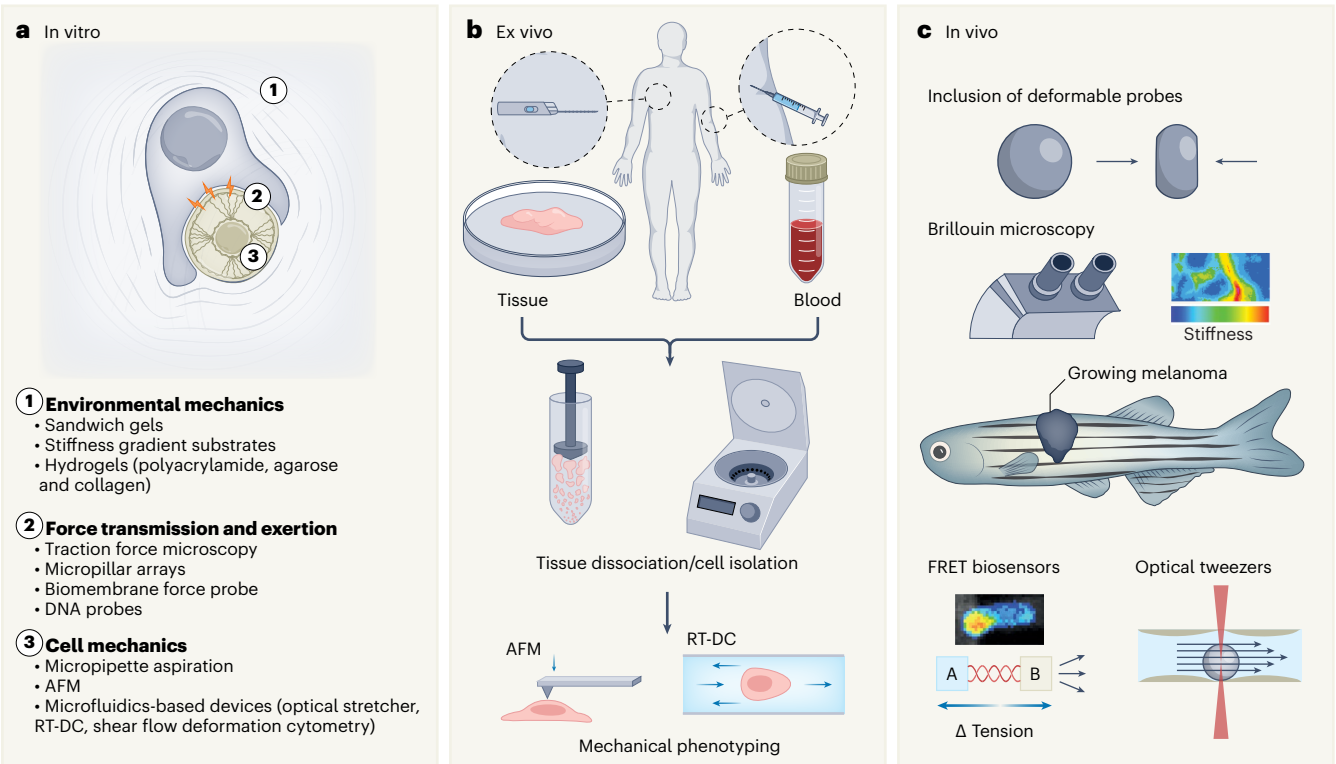
BOX 1

Relevant technologies for mechanical immunoengineering

Full dissection of the mechano-surveillance mechanisms inevitably relies on accurate methods for measuring forces. A plethora of in vitro techniques, such as micropipette aspiration, atomic force microscopy (AFM) and biomembrane force probe, have been available for measuring the mechanical properties of single cells, tissues and interaction forces between cells[7,211]. Although extensively used to highlight differences in mechanical properties between healthy and diseased cells and tissues, these techniques suffer from low throughput. More recently, microfluidic approaches have been developed to address this issue and allow high-throughput mechanical single-cell measurements. The development of real-time deformability cytometry (RT-DC) now allows to probe instant deformation and elasticity of cells at high throughput[212] and should soon expand its potential to allow fast measurements of viscoelastic properties[213]. The techniques mentioned above are all perfectly compatible with ex vivo measurements of patient-derived samples.

While certainly more challenging, probing forces and mechanical properties in vivo has a bright future ahead owing to recent technological breakthroughs. Brillouin microscopy has emerged to allow extraction of elastic and viscous properties of living tissues[214] and has been applied for mapping mechanical properties of cancer nodules in three dimensions[215]. Particle-tracking microrheology has been used to compare how mechanical properties of normal versus carcinoma cells evolve in living mice[216]. Hydrostatic pressure of fluid-filled cavities can be directly measured via the insertion of pressure-measuring micropipettes. Such methods were initially designed to measure pressure inside blood vessels[217] and can now be applied to measure cytoplasmic pressure in single cells[218]. Optical tweezers have been used to quantify haemodynamic and adhesion forces in the zebrafish embryo model[219]. Slightly invasive methods based on inclusion of deformable probes such as microdroplets/microbeads can quantify the mechanical stress acting on cells within living tissues[220,221]. Likewise, a recently developed microparticle-based platform was injected into tissues, allowing the characterization of the force profile of individual T cells in vivo[222]. Förster resonance energy transfer (FRET) tension sensors provide mechanical stress quantification when coupled with intravital imaging. These molecular springs can be genetically encoded in living cells/tissues, which then emit fluorescence that reports the local mechanical tension[223,224]. Such sensors have notably been used to quantify intercellular tension within endothelial cell monolayers exposed to fluid shear stress[225]. Similarly, viscosity can be followed in vivo by using molecular rotors that will adjust their conformational states upon viscosity changes[226,227]. Finally, breakthroughs in in vivo mechanical measurements have benefited researchers and found applications in the clinic. Novel diagnostic tools such as acoustic radiation force impulse[53] or magnetic resonance elastography[228] have allowed the probing of mechanical properties of patient-derived tissues in the clinic. In addition, recent progress in tissue mechanical dissociation coupled with RT-DC approaches will allow rapid probing of mechanical properties of surgically removed cells. This will help clinical decision-making during extemporaneous histological analysis[229]. Fine-tuning, spreading and combining these novel techniques will allow researchers to measure and investigate mechanical forces in the context of immune surveillance at all relevant scales, from whole tissues to single force-bearing receptors at immune synapses.



Relevant technologies for mechanical immunoengineering. **a**–**c**, A wide range of techniques are available to researchers for investigating environmental mechanics, interaction forces and cell properties in vitro (**a**), ex vivo (**b**) and in vivo (**c**).

antigen recognition, T cells form a well-defined nanostructure at their interface with antigen-presenting cells, called the immunological synapse (IS). T cells and their target cells are known to engage in extensive mechanical exchanges at the IS, which have been possible to investigate through the development of multiple nanotechnologies (see Box 1 on techniques). Both mouse and human $CD4^+$ T cells are activated to higher extents and secrete more effector cytokines when seeded on stiff substrates[8–11]. T cells exert mechanical forces to potentiate the killing of cancer cells[12]. In addition, T-cell-mediated lysis of tumour cells is enhanced when cancer cells are stiffened and decreased when they are softened[13–15]. These results provide solid evidence that mechanical aspects of pathologies do indeed impact immune responses.

Currently, most immunotherapies target biochemical cues of diseased tissues and immune cells. However, the growing appreciation of the mechanobiology of immune cells offers a good foundation for developing new engineering strategies aiming at mechanically modulating immune cells or target cells for enhanced therapies against various diseases. For example, emerging studies have been aiming at stiffening cancer cells to sensitize them to immune surveillance[14,15]. Expanding this concept to engineering strategies focusing on the mechanical modification of immunity–disease interactions shows enormous promise for developing next-generation immunotherapies. Here we first review the mechanical changes during pathological progression in several diseases, including cancer, fibrosis and infection. We then highlight the mechanosensing abilities of immune cells and the mechanosensitive nature of the immune response in various pathologies. Finally, we discuss how targeting the mechanical features of the disease milieu and immune cells is a viable strategy to enhance current immunotherapies. We also highlight several challenges yet to be addressed in this emerging field of 'mechanical immunoengineering'.

Altered mechanics in disease initiation and progression

Cancer

Mechanical changes in tissues constitute a signature trait of solid tumour pathologies that has long been exploited for diagnosing diseases, notably in breast cancer cases where mechanical probing of the tissues is routinely performed (palpation, mammography or elastography) (Fig. 1a). The mechanisms underlying the stiffening of tumour tissues and their metastatic secondary foci are well understood and have been expertly reviewed in the past[5]. Increases in the synthesis of matrix proteins, collagen crosslinking, integrin clustering and focal adhesion are the main contributors to extracellular matrix (ECM) remodelling. This is caused mainly by the concerted action of stromal cells and tumour growth-mediated solid stress, which leads to the stiffening of cancer tissues[16–18]. The pro-inflammatory environment of the initial malignant progression also sustains ECM stiffening. More specifically, cytokines and chemokines released by innate immune cells act in a paracrine way on stromal cells, driving their differentiation into 'activated fibroblasts' bearing a myofibroblast-like phenotype[19]. These 'activated fibroblasts', collectively named cancer-associated fibroblasts (CAFs), express higher levels of α-smooth muscle actin and collagen, and acquire greater contractile and proliferating capacities. Deposition of a stiff and non-elastic ECM in cancer results in impeded diffusion of chemotherapeutic molecules[20,21] and hinders infiltration of immune cells[22], which are key features of malignant progression. Other noteworthy changes during this desmoplastic reaction include disorganized and leaky vascularization. This results in a subsequent imbalance of interstitial fluid pressure, further preventing efficient delivery of chemotherapeutic molecules to the tumour[23].

Surprisingly, the stiffening at the scale of cancer (or metastatic) tissues can hardly be a representation or a consequence of the stiffness level of individual tumour cells. Although tumour tissues are stiffer than healthy tissues, it is so far widely accepted that tumour cells are softer than their healthy counterparts[24]. Consistently, nanomechanical profiling of tumour tissues has shown intratumoural stiffness to be highly heterogeneous compared with healthy tissues, with tumour cells constituting the softest spots of the probed cancer tissues[25]. As such, two very distinct scales must be considered when discussing mechanical changes in cancer diseases: (1) the whole-tissue scale, where solid cancer tissues, in general, are known to be stiffer than healthy tissues, and (2) the single-cell scale, where individual tumour cells are often thought to be softer than healthy cells. However, whether the consensus on the softer mechanical phenotype of single cancer cells is truly relevant remains debatable, as proper in vivo demonstrations have been lacking. As such, the potential link between the mechanical properties of tumour cells and disease progression remains unclear, despite some evidence hinting that both parameters probably affect each other. In addition, stromal components of tumours (fibroblast and immune cells mostly) are active elements from a mechanical standpoint and contribute to physical aspects of tumour progression, whose targeting offers a promising future for cancer therapy[26].

We recently speculated that cancer cells are not simply 'softer' but rather mechanically 'dynamic and adaptable', a feature that would undoubtedly grant them an important advantage for completing the metastatic cascade[27]. Accumulating evidence suggests that the mechanical properties of tumour cells confer a selective advantage in almost all the steps of cancer progression. A study investigating the link between mechanical phenotype and tumourigenicity of cancer stem cells found that only soft cancer stem cells could lead to primary tumour initiation[28]. This suggests that the mechanical properties of tumour cells are critical at the earliest stage of cancer progression. Tumour cells that later find themselves inside a growing primary tumour are subjected to increasing compressive stress levels and proliferate more efficiently by becoming spherical and stiffening their cell cortex[29]. Cancer cells that subsequently become invasive and escape the primary tumour site are softer and are subjected to gene expression changes associated with the epithelial–mesenchymal transition[30]. Such a softer mechanical phenotype facilitates migration through the ECM, as this process often requires extensive cell and nucleus deformation[31]. During intravasation, circulating tumour cells (CTCs) face drastic environmental changes. As their diameter is generally substantially larger than that of normal circulating cells, such as leukocytes, CTCs are more prone to shear-stress-induced destruction in mechanically hostile blood flow[32]. To cope with haemodynamic forces, CTCs have been shown to undergo stiffening and improve their chances of survival[33]. Tumour cells might again get softer once they have stably arrested and require performing extravasation through diapedesis[34]. Such an example of a dynamic adaptation mechanism to perform extravasation has recently been observed for primordial germ cells in avian embryos[35]. How mechanical properties of tumour cells evolve after extravasation remains largely unknown at this stage. However, one study suggested that freshly extravasated pericyte-like tumour cells might stiffen in a yes-associated protein (YAP)- and myocardin-related transcription factor (MRTF)-dependent manner to facilitate colonization of perivascular niches[36]. Such post-extravasation mechanical adaptation may impact or be impacted by the state that tumour cells might enter next: dormant or proliferative. In conclusion, the mechanical plasticity of tumour cells is an additional hallmark of metastatic progression.

Viral and bacterial infections

Similarly to cancer, two scales must be considered when discussing mechanical changes associated with viral and bacterial infections (Fig. 1b). Indeed, at the intracellular scale, bacteria and viruses can hijack the cytoskeleton of host cells to enhance transportation to the cytoplasm where they will replicate and assemble. In addition, bacteria and viruses can promote local actin polymerization to generate a mechanical force facilitating their progression. This phenomenon applies to several viruses, such as coronavirus[37] and respiratory syncytial virus[38]. Cytoskeleton changes do not occur exclusively in the

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host cells infected by viruses (that is, angiotensin-converting enzyme 2-positive cells in the case of severe acute respiratory syndrome coronavirus 2), but also in the immune cells at the infection site. Indeed, a recent study showed lymphocyte stiffening due to the clinical syndrome of coronavirus disease 2019[39]. Like cancer cells, pathogens, and more specifically bacteria, can also be affected by fluid shear stress[40,41] and develop strategies to resist it[42].

At the whole-tissue scale, bacteria and viruses can alter the biophysical properties of the infected tissue by changing the ECM structure and/or composition. Upon viral infection by rhinovirus, stromal cells markedly increase their production and deposition of fibronectin, perlecan and collagen IV in response to activation of Toll-like receptor (TLR)-3 and -7/8[43]. In parallel, TLR activation in stromal and epithelial cells triggers the secretion of high-molecular-weight hyaluronic acid[44], which traps fluid arriving through permeable endothelium and swells tissue, causing oedema and increased stiffness[44]. Oedema formation can be further fostered by secreted matrix metalloproteinases that degrade the endothelial barrier, resulting in abnormal Starling forces and fluid diffusion[45]. Upon acute inflammation, the lymph node architecture may undergo a late remodelling process, resulting in increased collagen deposition and ECM remodelling, which can profoundly impact tissue stiffness, such as scarring and eventually fibrosis[46]. Mechanical changes are actively experienced by immune cells as they travel through different tissue environments and contribute to the early recognition of danger and the induction of immune responses.

Fibrosis

Fibrotic diseases, such as idiopathic pulmonary fibrosis or scleroderma, are accompanied by numerous mechanical alterations (Fig. 1c). Fibrosis is the wound-healing response following iterative injuries of chemical (asbestos, alcohol and so on), mechanical or microbiological (hepatitis viruses, *Mycobacterium tuberculosis* and so on) origins[47,48]. Regardless of the underlying cause, chronic injury causes parenchymal cell death associated with a positive feedback loop between inflammatory damages, fibroblast proliferation and matrix deposition[49]. In some cases of acute stress, such as myocardial infarction, the emergence of fibrotic scars is crucial to replace the necrotic tissue and avoid cardiac rupture[50].

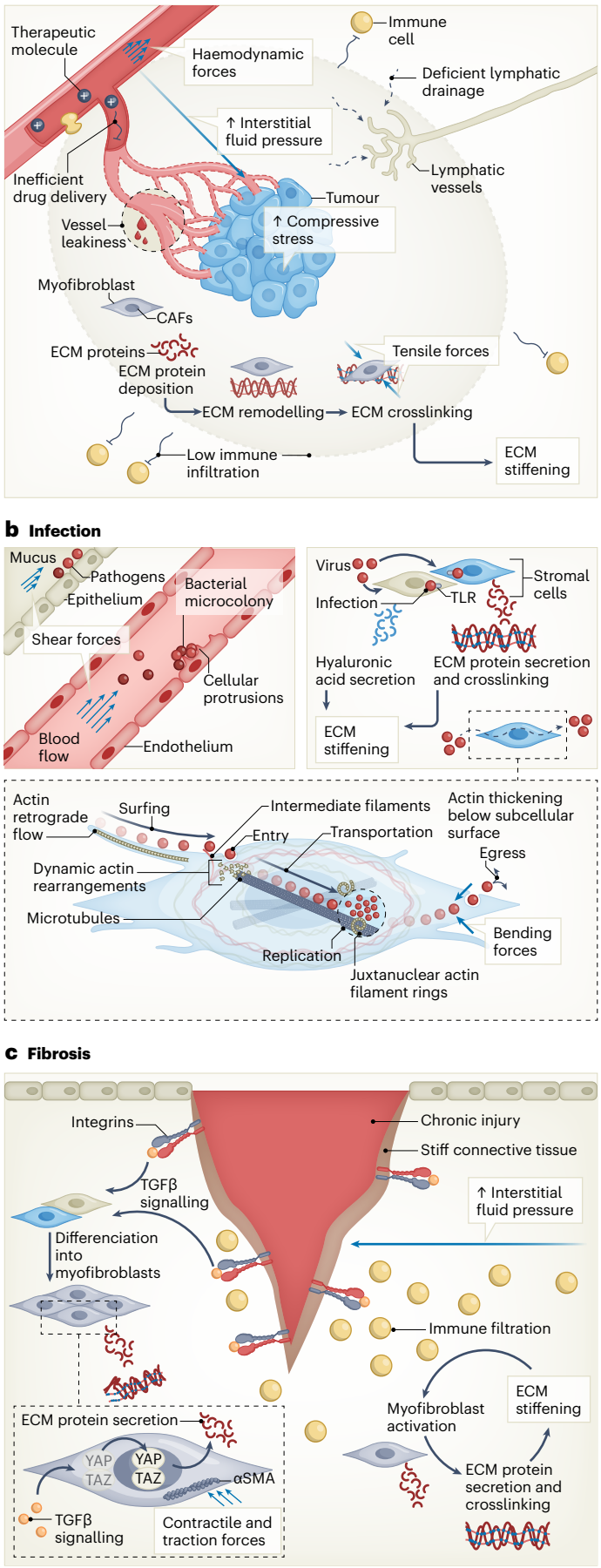

Fig. 1 | Different disease settings share common mechanical cues. a, Solid cancer diseases come with a variety of mechanical features that lead to the characteristic stiffness of tumour tissues. Compressive stress arises as the tumour volume expands and interstitial fluid pressure increases because of vessel leakiness and inefficient lymphatic drainage. Activated CAFs exert tensile forces on ECM fibres that they also secrete, remodel and crosslink. As a result of these changes, immune infiltration and delivery of therapeutic molecules to the tumour site are compromised. **b**, Mechanical forces are also at play in infectious diseases. Pathogens are sensitive to shear forces they encounter in mucus as they attempt to cross an epithelium or in the blood flow. Bacteria form microcolonies to deal with such forces. Stromal cells infected by viruses drive ECM stiffening by secreting hyaluronic acid and ECM proteins upon TLR activation. At the intracellular level, viruses hijack the cytoskeleton of their host cell to complete their replication and thrive. Viruses take advantage of actin retrograde flow in protrusions to 'surf' to their entry point. Entry is allowed by actin rearrangements and intermediate filaments acting as co-receptors at the cell surface. Microtubules are exploited for transportation to the replication site where acid filament rings come into play. Finally, actin thickening below the cell surface generate the bending forces necessary for freshly replicated virus particles to exit. **c**, Fibrotic diseases share very similar traits to cancer. Integrin-mediated TGFβ signalling originating from stiff connective tissue causes stromal cells to differentiate into activated myofibroblasts that secrete and crosslink ECM fibres and also exert high α-smooth actin-driven contractile and traction forces. The resulting ECM stiffening further activates myofibroblasts, thus creating a feedback loop that endlessly drives cellular activation and fibrosis. In addition, the chronic inflammation state of fibrotic tissues also leads to immune and fluid infiltrations that increase local hydrostatic pressure and further contribute to the stiffening of fibrotic tissues. α-SMA, α-Smooth muscle actin (α-SMA).

Increases in collagen and glycosaminoglycan depositions in the ECM promote the development of non-contractile fibrotic areas, leading to progressive tissue stiffening, which is a hallmark of fibrotic diseases. Indeed, various studies have shown that the elastic modulus of lung[51], liver[52] and kidney[53] tissues increased upon fibrosis when compared with healthy tissues. Several diseases, such as myocardial infarction, inflammatory cardiomyopathy or non-alcoholic fatty liver disease, can evolve into fibrosis[48,54]. A chronic inflammatory state can also occur with ageing. Indeed, unresolved systemic inflammation is a hallmark of immune ageing[55]. This persistent low-grade inflammation can lead to arteriosclerosis characterized by a stiff and thickened artery wall, resulting in the decline of cardiac function[56].

Although more work is needed to understand how matrix stiffness regulates tissue fibrosis comprehensively, accumulating evidence indicates that stiffening of connective tissue directly contributes to the progression of the fibrosis by promoting three fundamental profibrotic mechanisms: (1) mechano-activation of myofibroblasts through mechanotransduction pathways, (2) integrin-mediated activation of latent transforming growth factor β1 (TGFβ1), and (3) activation of non-canonical TGFβ1 signalling pathways. Mechanically induced activation triggers myofibroblast secretion of multiple types of ECM protein as well as matrix-crosslinking enzymes, resulting in a more stable and stiffer ECM[57]. This effect results in a positive feedback loop, leading to persistent cellular activation and fibrosis[58]. Moreover, reinforced activation of myofibroblasts also leads to upregulation of α-smooth muscle actin, further increasing their contractility[59]. As a result of this hypercontractile state, myofibroblasts exert higher traction forces on the stiff fibrotic ECM, which leads to more matrix remodelling and stiffening and integrin-mediated latent TGFβ1 activation (integrin αvβ6, αvβ5 and αvβ1), thus completing the fibrosis amplification loop[60]. Consequently, matrix stiffening in fibrosis should not simply be viewed as a by-product but as a key initiator and regulator of profibrotic fibrogenesis[61].

Mechanosensing and mechanotransduction of the immune system

Immune cells respond to various biochemical signals arising from pathogen-associated molecular patterns or specific activating ligands expressed at the surface of infected or transformed cells[62]. These signals are first detected by sentinel cells of the innate immune system, such as macrophages, dendritic cells (DCs) and natural killer (NK) cells. The innate immune system recognizes biochemical signals quickly through germ-line-encoded pattern-recognition receptors (PRRs) and other cell surface molecules, orchestrating inflammatory responses[63]. The effector functions of innate immune cells further drive adaptive immune responses by recruiting and activating T and B cells[63]. Professional antigen-presenting cells (APCs) expressing co-stimulatory molecules (CD80, CD86 and so on) prime T cells by presenting them cognate antigens as peptides bound to major histocompatibility complex (MHC) molecules[64].

In addition to their biochemical activation, immune cells encounter numerous forces as they circulate throughout the body to perform immunosurveillance and effector functions. Accumulating evidence shows that immune cells are sensitive to mechanical stimuli, such as shear stress, hydrostatic pressure and tension of their surrounding environment. Such forces are sensed and converted from mechanical cues to biochemical signals, thus activating cellular pathways and influencing their function. Current understanding of the mechanosensing of immune cells has been enabled largely by in vitro models of ligand-coated artificial substrates (for example, polydimethylsiloxane (PDMS), polyacrylamide gels, beads) that allow the decoupling of biophysical cues and biochemical signals involved in the interactions of immune cells with their environment and target cells[65,66]. As the physical properties of their surrounding microenvironment are essential to immune cell function, PDMS hydrogels offer an ideal model to manipulate and test the impact of stiffness[67]. Yet, the stiffness of PDMS gels typically spans from 100 kPa to 10 MPa (ref. 68), whereas the physiological stiffness normally ranges from 100 Pa (brain) to 100 kPa (carcinoma), except bones[69]. Therefore, polyacrylamide gels (typical stiffness ranging from 10 kPa to 10 kPa) might be more suitable to recapitulate the physiological stiffness of tissues[70]. Nevertheless, efforts are needed to generate models that better mimic the dynamic mechanical environment and cellular stiffness to model cell–cell interactions.

Monocytes and macrophages

Monocytes and macrophages are important innate immune cells that have a pivotal role in the initial recognition and elimination of pathogens through phagocytosis and/or cytokine secretion. Monocytes circulate in the peripheral blood for approximately one to three days and then migrate into tissues throughout the body where they differentiate into macrophages and DCs[71]. As these cells scout the body using body fluids, they are subjected to hostile fluid forces. For example, patrolling monocytes can sense haemodynamic forces, such as fluid shear stress, through mechano-gated ion channels[72,73]. The responses of circulating monocytes to high shear stress include enhanced adhesion, activation of CD11b integrin, phagocytosis and pro-inflammatory cytokine secretion[74].

Macrophages are terminally differentiated monocytes that reside in tissues, actively sensing their surroundings and launch effector responses accordingly. Macrophages are markedly affected by the wide range of phagocytic targets (pathogens, cancer cells, apoptotic bodies and so on) and their mechanical parameters[75–77]. Macrophages sense opsonized target stiffness through the Fcγ receptor, triggering integrin localization into the phagocytic cup. This further allows macrophages to close the phagocytic cup and complete phagocytosis[78]. Furthermore, target stiffness can overpower the signal-regulatory proteinα (SIRPα)-CD47 'don't eat me' signal[76]. In addition to phagocytosis, substrate stiffness is instrumental in tuning macrophage migration[77] and differentiation into anti-inflammatory ('M2 like') and pro-inflammatory ('M1 like') phenotypes[77]. Multiple research groups have shown that macrophages cultured on soft substrates have reduced inflammatory activation compared with those cultured on stiff surfaces[77]. Macrophages can sense their substrate's mechanical properties through integrins[79] and ion channels, such as PIEZO1 and transient receptor potential vanilloid 4 (TRPV4). The stiffness of the substrate favours the influx of Ca^{2+} ions through PIEZO1, a mechano-gated ion channel, and subsequent nuclear factor kappa B (NF-κB) activation and secretion of inflammatory cytokines leading to a more prominent 'M1 like' inflammatory phenotype[79].

Furthermore, the concerted action of PIEZO1 and TLR-4 is necessary to achieve innate responses against pathogens, including phagocytosis, reactive-oxygen-species production and bacterial clearance[80]. In addition to ion-channel-mediated mechanosensing, yes-associated protein and its functional partner, the transcriptional co-activator with PDZ-binding motif (YAP/TAZ) signalling orchestrates the response of macrophages to biophysical cues. Indeed, the translocation of YAP/TAZ to the nucleus, which allows the regulatory activity of downstream gene expression, is impacted by ECM stiffness[81]. When modelled in vitro, the stiffness of the culture substrate regulates the nuclear translocation of YAP and its downstream functions in various cell types[82–84]. In particular, macrophages cultured on hydrogels with a stiffness of 20 kPa or more have increased nuclear YAP, enhancing their response to the inflammatory agonist lipopolysaccharide[84].

Dendritic cells

DCs are the dominant population of professional APCs that present antigens to naive T cells and B cells[85]. Activation of DCs by their endogenous PRR or other inflammatory mediators leads to the overexpression of specific activation markers, such as CD80, CD83, CD86, and MHC class I and class II. In addition to biochemical stimuli[86–88],

DCs sense mechanical cues through ion channels, integrins and the YAP/TAZ pathway, influencing DC biology (migration, antigen uptake, IS formation)[88–90]. Shear stress activates mechano-gated ion channels of circulating DCs, inducing high expression of activation markers MHC class I and CD86[91] and forming podosomes, which improve their adhesion and migration abilities[91,92]. Once infiltrated in the inflamed stiff tissue, DCs adjust their activation levels and cytokine secretion. DCs cultured on stiff substrates show high YAP/TAZ nuclear translocation, leading to enhanced expression of downstream genes regulating DC activation, co-stimulatory molecule (that is, CD80/CD86) expression, proliferation and metabolism[93]. In addition, DCs modulate their antigen internalization and presentation capacities according to substrate stiffness[93,94]. A partial explanation can be found in the recent description of substrate stiffness responsiveness of the C-type lectin receptors, which are essential for antigen internalization by DCs[94,95]. Other studies show that DC activation through antigen uptake increases their cortical stiffness without necessarily increasing the expression of their maturation markers[96]. Thus, increased DC cortical stiffness is an additional feature of maturation that further lowers the antigen concentration threshold needed for efficient T-cell activation[11]. Indeed, actin cytoskeleton changes in mature DCs restrain the intercellular adhesion molecule (ICAM) lateral mobility, which promotes T-cell priming via affinity regulation of the lymphocyte function-associated antigen 1 (LFA-1) expressed by interacting T cells[97]. Finally, stiffness of the environment impacts mechano-gated ion channels that regulate the secretion of polarizing cytokine interleukin-12 (IL-12) by DCs, resulting in T helper cell 1 (T_H1) differentiation, inhibition of regulatory-T-cell lineage commitment and tumour growth inhibition[98].

Lymphocytes (T cells, NK cells and B cells)

Upon scouting throughout the body, lymphocytes recognize characteristic biochemical features that lead to their activation. Lymphoid cells also encounter different environments in which they can sense mechanical cues through mechanosensitive receptors, including integrins[99,100], ion channels (for example, TRPV4, PIEZO)[101], the T-cell receptor (TCR)[102,103] and cytoskeletal components[104,105], and convert these biomechanical stimuli to biochemical signals (migration, scanning, activation and so on). Emerging evidence shows mechanical cues govern $CD4^+$ and $CD8^+$ T-cell activation and effector functions. When seeded on stiff artificial substrates presenting CD3 and CD28 antibodies, mouse and human naive $CD4^+$ T cells show higher proliferation, metabolism and cytokine production[8,10,106]. TCR-related ZAP70 and Src kinases are phosphorylated in response to mechanosensing[8,107]. The stiffening of professional APCs also seems to enhance $CD4^+$ and $CD8^+$ T-cell activation[11]. Upon activation, the TCR not only senses mechanical cues of its environment but also exerts active pulling forces against their antigen[102,108,109]. DNA-based tension sensors (see Box 1 on techniques) revealed that T cells harness cytoskeletal coupling to transmit 12–19 pN forces per TCR, initiating strong T-cell activation[103,110]. Co-stimulation by CD28 further increases cellular force generation on TCR adhesions[108]. Moreover, TCR activation induces the inside-out upregulation of integrins, providing synergistic adhesive strength and mediating force transmission at the IS[111–113]. Other mechanoreceptors, such as PIEZO1, also synergize with TCR mechano-activation[114]. Mechano-activation of lymphocytes by TCR signalling also triggers cytoskeleton changes, inducing cytotoxic granule polarization and IS formation[111,112,115,116]. Besides, cytoskeleton disruption by pharmacological small molecule inhibitors (for example, latrunculin A, blebbistatin, nocodazole) completely abolishes immune cell stiffness-dependent activation and cytokine secretion[14,15,117]. In that way, cytoskeleton changes act as a second layer of mechanosensing by T cells. While naive T cells have a stiff cortical cytoskeleton, effector T cells are typically softer, resulting in the formation of larger IS with APCs[104]. This observation suggests that in addition to TCR mechanosensing and force exertion, the baseline cytoskeletal state controls T-cell responsiveness.

Similarly, NK cells sense the mechanical features of their environment that synergize with biochemical signals to modulate their activation and inhibition[11,66,118,119]. Stiff substrates coated with antibodies directed against LFA-1 or the natural cytotoxicity receptor NKp30 stimulate NK-cell degranulation by recruiting phosphorylated ZAP70 to the NK IS[120,121]. Stiff substrates also induce strong clustering of DNAX-protein 10, whose hexametric complex with NKG2D activates signalling in NK cells[66,122]. On the contrary, softer substrates impair microtubule organizing centre polarization, lytic granule polarization and F-actin accumulation at NK IS, leading to the formation of unstable asymmetrical synapses and decreased degranulation[123]. How NK cells sense the mechanical features of their environment is still unclear, but a recent study leans towards mechano-gated ion channels[124].

Like T and NK cells, B-cell activation is also modulated by the mechanical properties of their environment and partners, notably by the stiffness of the APCs[125]. Antigens presented on stiff substrates induce higher B-cell receptor microcluster formation compared with antigens tethered to soft substrates, leading to increased B-cell activation[125]. In addition, B cells are also able to differentiate high-affinity antigens from low-affinity antigens tethered on the APC by using diacylglycerol kinase ζ and myosin IIa to pull-out and invaginate the APC membranes presenting high-affinity antigens[126,127]. Therefore, substrate stiffness has a crucial role in B-cell activation, affinity maturation, class switch and antibody responses[128,129]. Although stiff substrates have been reported to induce better B-cell receptor clustering, high-affinity antigen extraction and affinity maturation, soft substrates, on the other hand, appear to be preferable for class switch and T-cell-independent antibody responses[128]. Altogether, the physical extraction of environmental signals by B cells accelerates their adaptation but may also cause the extinction of unfitted cell populations[130].

Evidence and mechanisms of mechano-immune surveillance

Anti-infection mechano-immunology

Viruses and bacteria can modify the host mechanobiology at the single cell or the tissue levels to evade immunosurveillance. At the host-cell level, the mechanical evasion from immunosurveillance is often mediated by a remodelling of the actin cytoskeleton. For example, upon infection, the human cytomegalovirus expresses the pUL135 protein, inducing remodelling of the actin cytoskeleton. Such a process is characterized by actin stress fibre loss, which markedly reduces the efficiency of IS formation upon recognition by immune cells[131]. This trait is exploited by human cytomegalovirus to protect the host cell against cytotoxic immune effector cells and to promote viral proliferation. Likewise, bacteria can also impair IS assembly by either reducing antigen presentation by APCs in *M. tuberculosis* infections[132] or by increasing T-cell actin polymerization, thereby impairing the ability of T cells to scan APCs in *Shigella*-mediated diarrhoeal diseases[133].

Nevertheless, actin cytoskeleton modifications can eventually serve as damage-associated molecular patterns to activate the immune system. For instance, F-actin is recognized by the dendritic cell natural killer group receptor-1 (DNGR-1) specifically expressed in DCs, leading to cross-priming of cytotoxic T lymphocytes (CTLs) in virus-infected mice[134]. Actin polymerization is also required to induce the NLR family CARD domain-containing 4 (NLRC4) inflammasome and to secrete antimicrobial molecules by bone-marrow-derived macrophages[135].

While altered biophysical properties of infected cells lead to immune evasion at the single-cell scale, pathogen-infected tissues with altered mechanical properties at the tissue level induce potent immune activation. Early activation of PRRs in Langerhans cells and stromal cells activated by virus- and bacteria-derived danger signals triggers the secretion of copious amounts of hyaluronic acid to promote oedema and attract immune cells to the infection site[44]. Mechanical forces applied on immune cells when they squeeze through the endothelium and dense ECM drive chromatin changes that promote

inflammation and chemotaxis-related transcripts[136]. At the same time, oedema-induced tensile stress and hydrostatic pressure encountered by innate immune cells activate their mechanosensitive ion channels (PIEZO1, TRP and so on), priming them for bioenergetic demands of activation and reducing the threshold required for optimal PRR stimulation[80,93,137]. These ion channels play an important role in the early immune response against infections as their depletion abrogates the inflammatory response that clears pathogens[137,138].

Upon immune-response progression, T cells migrate and proliferate in the lymph node, which causes local stiffening and swelling[139]. Lymph node tissue mechanics can be sensed by T cells, which further promotes their proliferation, activation and metabolism while lowering the antigen dose needed to elicit effector responses[139]. Stiffness of the environment can be sufficient to prime T cells, as reported in a recent study. Indeed, T cells that crawl through stiff 40 kPa matrices show enhanced activation, regardless of the stiffness level of the APC that interacts with them[106]. Activated T cells will eventually migrate to an infection site where they will experience multiple mechanical stimuli. Notably, migrating T cells interact with secreted hyaluronic acid through CD44, a key mechanosensitive receptor involved in T-cell extravasation[140]. Additional studies will be necessary to decipher whether this interaction promotes or suppresses T-cell activation. Finally, mechanical changes in tissues are reverted during infection resolution, with dissolution of hyaluronic acid, reduced oedema and impaired immunity to danger signals. However, chronic inflammation can sometimes arise from a positive association of mechanical stimulation, T-cell-dependent T_H2 cytokine (IL-4 and IL-13) and chemokine (monocyte chemoattractant protein-1(MCP-1)) signalling, leaving a lasting mechanical imprint at the inflammatory response site[141].

Anticancer mechano-immune surveillance

Anticancer immune surveillance kicks in early after malignancy and the immune system patrols throughout the body to identify and destroy nascent tumours[142]. NK cells and CTLs recognize transformation-associated molecules and tumour-associated antigens expressed on the surface of tumour cells[143]. Upon formation of the IS between cancer cells and lymphocytes, lymphocytes secrete the pore-forming protein perforin, which allows entry of death-inducing toxic granzymes and lysis of target cancer cells[144]. Although tumour cell killing had been mostly linked to canonical signalling pathways involving receptor–ligand recognition, recent evidence demonstrates that lymphocytes also sense tumour-dependent mechanical cues (see the 'Mechanosensing and mechanotransduction of the immune system' section) and respond accordingly towards target cells. Upon tumour infiltration and IS formation, NK cells and CTLs exert up to nanonewton-scale cellular forces towards target cells[102,108,109]. The force exerted at the IS regulates the cytotoxic responses in several ways. Mechanical cues are transduced inside lymphocytes from the plasma membrane into the nucleus through the linker of the nucleoskeleton and cytoskeleton complex. This leads to the expression of target genes (for example, *CD69*, *IL2*, *IFNG*), lymphocyte activation and the formation of a mature IS[145]. Forces exerted at the IS also directly influence lymphocyte-mediated cytotoxicity by increasing membrane tension and potentiating lysis of target cells[12,15]. During this process, surface receptors (for example, TCR, $\alpha_L\beta_2$ integrin LFA-1, NKG2D) at the IS change their conformation to form catch bonds with their respective ligands. Such catch bonds were found to rely on forces to achieve high affinity for their ligands[103,146,147]. The forces exerted at the IS heavily depend on the target cell's mechanical profile and compliance. Hence, it is important to note that the biophysical properties of the targeted tumour cells profoundly regulate the killing processes[12]. We and others recently found that the softness of cancer cells, independently of other biochemical features, acts as an 'immune checkpoint' of mechanical nature (or termed 'mechanical immune checkpoint'), which induces immune evasion of lymphocyte-mediated cytotoxicity[13–15]. The immune system is consequently more sensitive to stiff(er) tumour cells, yet the mechanisms must be fully unravelled (Fig. 2). First, stiff cancer cells may allow higher synaptic force exertion by lymphocytes, which potentiates cytotoxicity by enhancing target cell membrane tension to facilitate pore formation mediated by perforin[12,15]. Second, the mechanics of target cells probably impact the strength and morphology of the IS. Lymphocytes spatiotemporally coordinate the force exertion and perforin secretion within the synapse by forming Wiskott–Aldrich-syndrome-dependent interfacial actin-based protrusions[112,148,149], which could be enhanced when encountering stiffer target cells. Third, stronger activating signalling through lymphocyte's mechanosensitive receptors exerted by stiffer target cells could also increase the degranulation and cytokine production, leading to enhanced cytotoxicity[14,15]. Once cancer cell killing is achieved, immune cells sense the cytoskeletal contraction of apoptotic targets and respond to this mechanosensory feedback by dissolving the IS. Such IS turnout is critical for serial killing by CTLs and apoptotic body clearance by patrolling phagocytes[150]. Altogether, immune cells, particularly CTL and NK cells, modulate their activation and cytotoxic functions according to the biophysical status of the cancer cells that they probe. This biophysical angle of immunosurveillance has led to the emergence of a new unique field of 'mechano-immune surveillance'.

Several strategies have been reported to stiffen cancer cells for therapeutic purposes. Cholesterol enrichment in the plasma membrane reduces the cortical (plasma membrane and the underlying dense actin network) stiffness of cancer cells[151,152] in many types of cancer. One can thus envision that stiffening tumour cells by depleting membrane cholesterol with methyl-β-cyclodextrin (MeβCD), a cholesterol scavenger, could be a promising therapeutic intervention[15]. Treatment with MeβCD efficiently decreased the cholesterol levels at the plasma membrane of mouse and human cancer cells. It further increased the target cell stiffness, and sensitized the cancer cells towards T-cell-mediated killing in vitro and in vivo. Impressively, the combination therapy of MeβCD and adoptive transfer of tumour-specific T cells adjuvanted with an IL-15 super-agonist (IL-15SA) increased the rate of complete tumour eradication to 41.7% compared with 0% in the case of immunotherapy alone. In another elegant study, a subset of metastatic cancer cells was found to overexpress MRTFs, a cytoskeletal-associated transcriptional factor known to promote cancer migration and metastasis[36].

Interestingly, MRTF overexpression also enhances F-actin polymerization and, thereby, the stiffness of cancer cells. By inducing MRTF overexpression, the cancer cells increase their membrane tension, becoming more susceptible to T-cell and NK-cell-mediated cytotoxicity due to increased degranulation and cytokine production[14]. Of note, the selective depletion of F-actin in cancer cells completely abolishes the immune sensitization induced by MRTF overexpression, confirming the biophysical basis of these observations. Similarly, treating cancer cells with jasplakinolide, an actin polymerization promotor, also increased the stiffness of tumour-repopulating cells, a specific population among tumour cells that show features of self-renewal, low differentiation, high tumourigenicity and, in particular, softness and resistance towards T-cell-mediated killing[13]. Therefore, the combination of jasplakinolide, adoptive T-cell transfer therapy and programmed cell death protein 1 (PD-1) antibody improved the therapeutic efficacy in a mouse melanoma model.

Towards mechanical immunoengineering strategies to improve immunotherapies

Targeting mechanical adaptability of tumour cells

As discussed above, antitumour mechanical immune checkpoints can be targeted therapeutically. Indeed, soft cancer cells can be stiffened and sensitized towards current immunotherapies, including immune checkpoint blockade antibodies and adoptive T-cell transfer[13–15] (Fig. 3). Such discovery is also clinically relevant as soft human cancer cells also show resistance to perforin-mediated killing[13].

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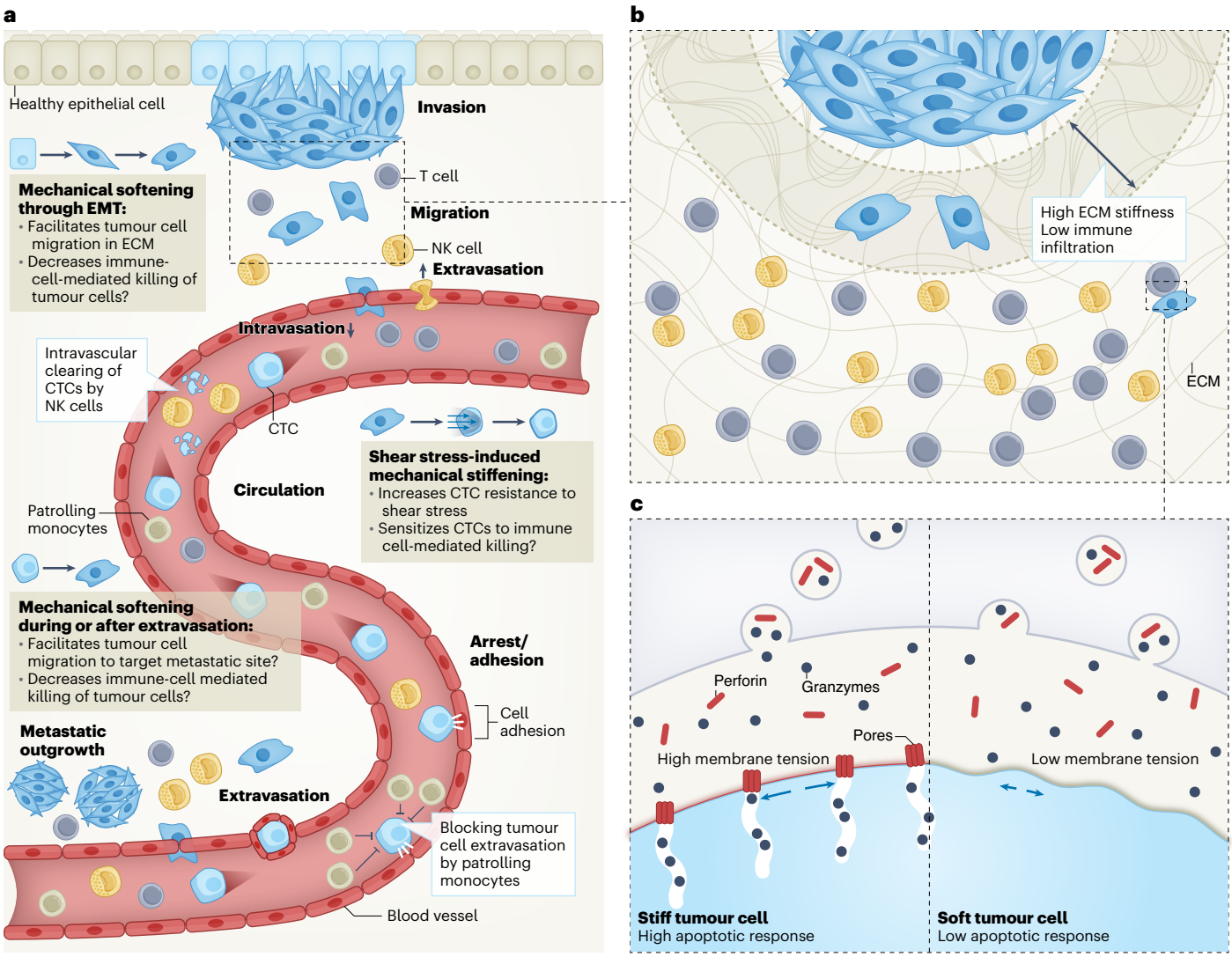

Fig. 2 | Spatiotemporal windows for optimal mechanical antitumour activity of immune cells. a, Tumour cells are probably adjusting their mechanical properties as they progress through the metastatic cascade. Following transformation of healthy epithelial cells and rise of a primary tumour, a select number of cancer cells will get softer as they go through the epithelial–mesenchymal transition (EMT) and perform invasion and migration. They later undergo stiffening as they enter the blood flow and become CTCs. As such, taking advantage of the optimal time windows when interactions between immune cells and stiffened tumour cells are most likely to occur might be key when establishing therapeutic strategies. Examples include NK cells performing intravascular clearing of CTCs and patrolling monocytes blocking tumour cell extravasation. **b**, Alternatively, windows in which immune cells show shortcomings could be identified to leverage mechanical immunoengineering and help them overcome them. The primary tumour site with its stiffened ECM and softened tumour cells represents a good example of such windows, with suboptimal mechanical features for NK- and T-cell antitumour activity. **c**, The aim should be to get immune cells in contact with tumour cells as stiff as possible as increased target cell stiffness favours IS formation and potentiates perforin and granzyme-dependent cytotoxic activity of immune cells.

Moreover, patients with melanoma exhibiting constitutive actin cytoskeleton-mediated stiffening are found to present enhanced responsiveness to immune checkpoint blockade therapy[14]. Yet, tumour cell mechanics cannot be a binary process where softness would fully explain and correlate with malignancy and metastatic progression. On the contrary, we believe that cancer cells are 'dynamic and adaptable' to perform and survive through the multiple mechanical and biochemical constraints they face along their path to metastasis[27] (as discussed in the 'Altered mechanics in disease initiation and progression' section). Their mechanical plasticity is an additional hallmark of metastatic progression that is likely to impact mechano-immune surveillance with, in theory, spatiotemporal windows in which immune cells can most efficiently recognize and kill tumour cells. As CTCs may get stiffened in response to the hostile haemodynamic forces in the circulation, we expect them to be, at this moment, the most vulnerable to T- and NK-cell-mediated killing, whose efficiency is likely also impacted by shear forces[101].

Recent studies have reported that CTCs could indeed be sensitive to immune cell-mediated killing, which contributes in part to the inefficiency of the metastatic process[153–155]. One of them showed that NK cells could clear tumour cells in the intravascular environment around the liver site and limit tumour cell seeding at a distant site in the lung. That same study highlighted an efficient synergy between NK cells and T cells, with the NK cells killing tumour cells in circulation and promoting T-cell recruitment, which would then restrict metastatic foci growth in situ[154]. In addition, patrolling monocytes might also contribute to antitumour activity in the circulation by directly scavenging tumour materials, secreting cytokines[156] and recruiting NK cells[153,157]. While evidence of intravascular antitumour activity of immune cells exist, this metastasis stage appears challenging to exploit because of the highly

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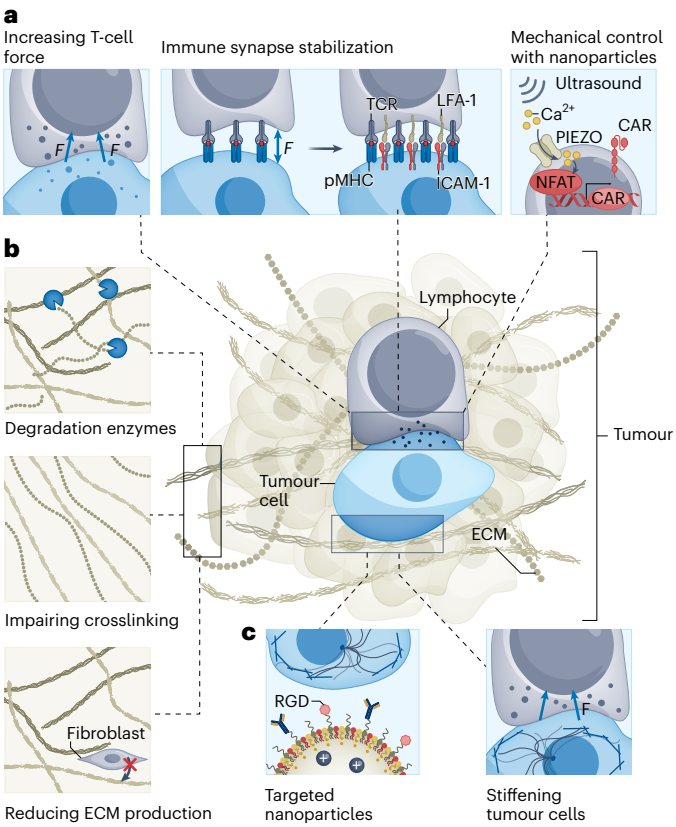

Fig. 3 | Improving therapies through mechanical immunoengineering of tumours and immune cells. Immune cells, tumour cells or the ECM of the tumour environment can be targeted using different strategies aiming at modulating the mechanical features of the immune response. **a**, Potentiating the mechanical activity of T cells (grey) can be achieved by enhancing their force exertion abilities (F), increasing the stability of immune synapses composed of the TCR (blue) recognizing tumour-associated antigens presented by MHC class I (pMHC, dark grey) and integrins, or controlling spatiotemporal activation of key genes through novel tools (for example, microbubbles and ultrasounds activating genes expression under the nuclear factor of activated T-cells (NFAT) promoter). **b**, Inducing ECM remodelling through release of degradation enzymes, impairing ECM crosslinking with inhibitors, or decreasing ECM protein production in fibroblasts can improve immune cell infiltration to target disease sites. **c**, Altering the mechanical properties of tumour cells (blue) to increase force transmission at the IS and potentiate antitumour activity of immune cells can be achieved by using stiffening agents such as MeβCD, or antibodies/arginylglycylaspartic acid (RGD) targeted nanoparticles loaded with anticancer drug or gene therapy.

dynamic movement and low frequency of tumour antigen-specific T cells in the circulation[158].

Instead, stiffening tumour cells during the invasion and migration processes, at which stage they typically display soft mechanical phenotypes, may be a promising strategy to sensitize them to T- and NK-cell-mediated killing. Enhancing cancer cell stiffness could also favour macrophage engulfment as the target stiffness could partially override the SIRPα-CD47 'don't eat me' immune checkpoint[76]. Previous studies have shown that depleting membrane cholesterol using MeβCD[15], activating myosin with 4-hydroxyacetophenone (4-HAP)[159] or inducing actin crosslinking with jasplakinolide[13,28] can stiffen cancer cells. However, none of these pharmacological approaches are specific. A challenging aspect of such strategies is to find ways to specifically stiffen the tumour cells without stiffening the ECM, which would hinder immune cell infiltration, possibly trigger pro-metastatic mechanotransduction in tumour cells and all-in-all be counterproductive. Tumour-targeted-nanoparticles might be a promising tool for achieving specific stiffening of tumour cells[160]. Moreover, their own mechanical properties can be tuned to foster their internalization in tumour cells. Soft nanomaterials and microparticles tend to better accumulate in tumour tissues and be preferentially taken up by cancer cells[161,162]. In a recent example, anticancer drug-loaded nanoparticles were developed to target soft cancer stem cells, allowing their specific elimination in vitro and in vivo with minimal side effects[163]. Besides, recent studies have also demonstrated that cancer cells can be transiently stiffened through increased F-actin polymerization upon internalization of iron oxide nanoparticles[164]. Cytoskeleton alterations and cell stiffening upon nanoparticle internalization have been described in macrophages[165] and such an approach might also be applied to tumour cells. As an alternative mechanical intervention approach, gene therapies for overexpression of MRTF and acyl-CoA:cholesterol acyltransferase 1 might be promising to facilitate specific stiffening of cancer cells[14,15].

Targeting tumour ECM mechanics for enhanced immunotherapy

The tumour microenvironment (TME) is known to impair anticancer immune responses. In addition to the highly immunosuppressive biochemical signals in the TME[166], its biophysical properties play a crucial role in immune cell migration and activation. During malignant progression, increased ECM stiffness together with other biochemical changes (for example, integrin clustering, altered chemokine profile) alter infiltration of T cells and other anticancer immune cells, and restricts them away from tumour cell nests, which reduces their antitumoural capacities[22,167,168]. Factors including TGFβ, matrix components and integrins are known to control the mechanical properties of the tumour ECM and its sensing by cancer cells. In addition, recent studies have shed light on how ECM mechanical properties might impact tumour cell dormancy[169,170]. Therefore, targeting actors of ECM stiffness and improving the diffusion of chemotherapeutic drugs and/or immune cells is a therapeutic approach with clinical potential[171] (Fig. 3). Inhibiting the production of matrix components by fibroblasts can be achieved by blocking the TGFβ profibrotic pathway. The use of neutralizing antibodies or the repurposing of angiotensin inhibitors, such as Losartan, has been shown to efficiently reduce collagen and hyaluronic acid production[172,173]. Collagen fibre alignment can also be reduced by targeting the discoidin domain receptor 1 (DDR1) ectodomain with monoclonal antibodies[169]. This strategy restores T-cell invasion into the tumour bed, resulting in remission in 56% of treated mice bearing triple-negative breast cancer. Conversely, DDR1 has been described to sustain tumour cell dormancy by maintaining type III collagen fibre at a low degree of alignment[170]. Hence, DDR1 depletion and subsequent realignment of collagen fibres was found to be a key trait of the dormancy-to-reactivation transition[170]. Of note, this study also indicates that enriching the TME with collagen III could promote or even induce a dormant state, thus preventing the formation of metastases[170].

Hyaluronic acid can also be reduced in the tumour ECM by using degradative enzymes, such as the pegvorhyaluronidase alfa investigated in a phase III clinical trial in association with gemcitabine for patients presenting hyaluronan-high metastatic pancreatic adenocarcinoma (NCT02715804)[174]. However, the use of hyaluronidase might also degrade avascular cartilage. A hyaluronidase nanoformulation showed no accumulation at these sites[175]. When combined with gemcitabine to treat a pancreatic ductal adenocarcinoma mouse model, the hyaluronidase nanoformulation resulted in a near doubling of overall survival[175]. The safety of use can be further improved by developing TME-responsive nanoparticles. Reactive-oxygen-species-activatable collagenase is selectively activated in the TME, relieving ECM stress and increasing paclitaxel penetration[176]. Degradative enzymes of ECM components can be used to enhance adoptive cell transfer immunotherapies. One strategy relies on engineered chimeric antigen receptor (CAR)-T cells secreting heparanase, which degrades heparan sulfate

proteoglycans, one of the main components of the ECM[177]. These heparanase-secreting CAR-T cells showed improved ECM degradation capacity, which promoted immune cell infiltration in tumour xenografts, resulting in increased survival of treated mice[177]. Recently, new strategies based on the bioorthogonal modification of the surface of immune cells to avoid viral transfection have been developed[178,179]. CAR-T cells cultured with azide-containing saccharides allowed for metabolic incorporation and membrane expression of azide groups for further conjugation with dibenzocyclooctyne-modified hyaluronidase through click chemistry[180]. These hyaluronidase-modified CAR-T cells presented better infiltration in the tumour, which correlated with improved tumour regression and survival.

As an alternative strategy, ECM stiffness can be reduced by preventing matrix crosslinking. Lysyl oxidase (LOX) is an enzyme responsible for the reticulation of collagen fibres[17]. Its inhibition by β-aminopropionitrile allowed to overcome chemoresistance and increase intratumoural T-cell migration, thus improving response to anti-PD-1 therapy[181,182]. The co-encapsulation of LOX inhibitors and epirubicin resulted in better biocompatibility and prolonged survival, providing an all-in-one nanoparticle-based ECM-targeting chemotherapy for cancer treatment[183]. Nanoparticle-based approaches can also be used to assess LOX activity in the tumour ECM and allow selection of patients that may benefit from LOX inhibitor treatments in addition to their chemotherapy[184]. Other studies have shown that LOX antibodies functionalized on polymeric poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles efficiently decrease the expansion of breast cancer[185].

Finally, inhibiting fibroblast contractility may help to reduce global ECM integrity. Among the different targets, the use of fasudil to inhibit the Rho-associated protein kinase leads to loss of stress fibres and focal adhesion complexes[186]. Fasudil treatment, hence, alters cytoskeleton-based contractility of fibroblasts, disrupting ECM integrity and improving chemotherapy effectiveness[186]. Other studies have shown that focal adhesion kinases can be directly inhibited, reducing ECM force transmission to the actomyosin complex, and thus reducing cellular response to matrix mechanical cues. A focal-adhesion-kinase inhibitor (AMP945) improved response to gemcitabine in a pancreatic adenocarcinoma model and is now being investigated in a phase II clinical trial for this indication (NCT05355298)[187]. However, these molecules can lead to life-threatening adverse events, such as hypotension, hepatotoxicity and excessive bleeding. Efforts to develop targeted formulations of these molecules are still required to improve their safety in clinics. CAFs expressing the fibroblast activation protein (FAP) are found in nearly all solid tumours and can be depleted by using anti-FAP CAR-T cells. Several studies have evaluated the use of anti-FAP CAR-T cells, and it has been shown that depletion of FAP^+ stromal cells results in decreased tumour growth in an immune-dependent fashion[188,189]. Of interest, this strategy is also investigated to treat post-cardiac infraction fibrosis and to restore cardiac function[190]. While killing fibroblasts at the primary tumour site could reduce global ECM stiffness, their destruction in target organs of dissemination could help to maintain tumour cell dormancy[191]. Indeed, hepatic stellate cell activation upon chronic liver injury induces a myofibroblast-like phenotype that further disrupts NK-cell-sustained breast cancer dormancy[191]. As FAP is also expressed by skeletal muscle, adipose tissue and pancreas, depleting FAP^+ stromal cells can lead to muscle atrophy, bone marrow hypoplasia and rapid weight loss[192].

Strategies to soften the ECM have been extensively studied to enhance the diffusion of chemotherapeutic drugs into the tumour bed and could favour tumour targeting of other therapeutics, such as antibodies or CAR-T cells, whose access is also hindered by the stiff ECM[22,193]. Yet, only a few studies have investigated the perspective of ECM stiffness disruption to increase intratumoural T-cell migration and response to PD-1 blockade[169,177,180,182]. As ECM proteins cover a large part of the physical barrier hindering immune cell migration, strategies involving engineered CAR-T cells capable of degrading ECM proteins are elegant due to their abilities to degrade ECM while migrating and performing tumour cell killing. The therapeutic advantage of ECM softening in immune checkpoint blockage and cellular immunotherapies remains to be evaluated. Yet, due to the potential opposite functions of some targeted molecules, any therapeutic strategy aimed at influencing the ECM architecture will require careful planning and timing. Finally, combining these approaches with methods to control cell mechanics in an all-mechanics strategy, could synergistically enhance antitumour immune surveillance.

Mechanically enhanced T- and NK-cell-based immunotherapies

Most immunotherapies currently aim at targeting biochemical cues of tumours and immune cells to enhance the immune response. The emergence of the 'mechano-immune surveillance' concept paves the way for developing next-generation therapeutic strategies targeting biophysical features of immune cells to boost their functions against various diseases. Selective delivery to immune cells could be achieved by targeting selective immune markers with antibody–nanoparticle conjugates[160]. One such approach of mechanical immunoengineering could aim at enhancing the tumour infiltration of T cells. As described previously, tumour ECM presents a mechanical hurdle for antitumour immune cells to access the tumour bed[22,167,168]. One could circumvent that by further favouring the through-matrix migration potential of immune cells. For example, pharmacological or genetic manipulation of microtubules of T cells increases Rho-pathway-dependent cortical contractility, which favoured infiltration in a three-dimensional environment in vitro by shifting $CD4^+$ T cells towards an amoeboid phenotype[194]. In stark contrast, microtubule stabilization with a commonly used drug, taxol, induced a marked reduction in T-cell infiltration abilities.

Another strategy is to enhance the mechanosensitivity of immune cells. Mechanical force exertion and underlying actin remodelling are essential for IS formation and stabilization[109,112,115,116]. Indeed, treatment of T cells with the actin polymerization inhibitor latrunculin A resulted in complete abrogation of force exertion by T cells and consequent loss of activation and effector functions[12]. In contrast, modulation of actin polymerization-stimulating proteins at the lymphocyte/APC interface could provide a strategy for enhancing T-cell force exertion and thereby, possibly enhancing effector function and cytotoxicity. A series of studies showed that CTLs with phosphatase and tensin homologue (PTEN) knockdown show increased force exertion and enhanced tumour cell killing in vitro[12]. While CTL granule polarization and release were unaffected by PTEN knockdown, this strategy led to limited improvement of tumour growth control in vivo[195]. Nevertheless, the involvement of PTEN in numerous cellular processes makes it challenging for systemic inhibition of PTEN using pharmacological or genetic therapies, representing a key limitation in this strategy.

As an alternative approach, one may engineer T cells capable of developing a stable IS (Fig. 3). The canonical IS is characterized by a concentric architecture in which a central cluster of antigen-presenting receptors is surrounded by concentric, annular accumulations of integrins and F-actin[140]. In contrast to the IS of the TCR:peptide–MHC complex, the non-classical CAR IS shows less-organized structures with diffusive CAR:antigen clustering and reduced actin distribution[196]. As the IS structure and morphology influence the effector functions, CAR-NK cells have recently been engineered with a novel receptor that modulates and tunes the IS to a more ordered state[197]. Fusing a second-generation CAR with the post-synaptic density protein 95, *Drosophila* disc large tumour suppressor and zonula occludens-1 protein (PDZ) domain of a molecule implicated in IS formation, the cytotoxic and regulatory-T-cell-associated molecule, resulted in increased synaptic area and accumulation of pZAP70, a key kinase in T-cell signalling[197]. Moreover, the PDZ domain enabled additional scaffolding crosslinks between the CAR and the cytoskeleton via mediators such as ezrin.

Anchoring the receptor to cytoskeletal components enhanced the avidity and binding strength of IS thus succeeding in higher antitumour activity. CAR-T and CAR-NK cells were elegantly developed with synthetic Notch receptor (SynNotch) or apelin-based synthetic Notch receptor to increase their safety and expand the set of targetable antigens[198]. Activation of these receptors by their cognate ligand caused the proteolytic cleavage of their intramembrane domain, releasing a transcriptional factor that could activate a specific transcriptional programme (expression of a CAR, cytokines and so on)[199]. Structure-guided mutagenesis was also used to develop new cutting-edge designs of SynNotch receptors capable of increasing mechanical sensitivity to the piconewton range[200]. In response to varying tensile forces, these receptors are expected to enact tailored transcriptional programmes. These studies provide evidence that tuning the IS via anchoring domains or by repurposing SynNotch receptors to increase mechanosensitivity might offer an orthogonal and complementary dimension to biochemical engineering for CAR-based immunotherapies.

In addition, nanoparticles can be leveraged to improve immune cell activation. Several types of artificial APC composed of nanoparticles coated with anti-CD3, anti-CD28 or peptide–MHC complex have been developed[201] and can be used to trigger T-cell activation and expansion in vitro by TCR clustering under a magnetic field[202,203]. After adoptive cell transfer, these ex vivo-activated T cells showed better tumour control. Some ultrasmall silica nanoparticles can directly ligate the TCR:CD3 complex and activate the T cells[204]. As ultrasmall nanoparticles avoid liver accumulation and are quickly eliminated by renal clearance, they present very promising clinical translation prospects. A similar strategy has been evaluated for NK-cell activation. Magneto-activated NK cells show increased secretion of lytic granules correlating with therapeutic efficacies against hepatocellular carcinoma[205,206]. Other optomechanical actuators can be used to trigger immune cell activation such as optical systems[207] or ultrasounds[208]. However, these optical systems are poorly transferable in vivo due to the low light penetration through tissues (mirometres to millimetres)[209]. On the other hand, ultrasounds can penetrate tissues up to several centimetres. A sonic stimulus of integrin-bound microbubbles was used to induce calcium influx[210] through PIEZO1 channels in T cells in vitro. This was followed by the nuclear factor of activated T cells transcription factor translocation to the nucleus, which drove expression of T-cell activation genes. Despite the originality of this approach, in vivo translation is limited as shear forces will probably damage or burst the microbubbles coupled to T cells during their migration and extravasation.

Outlook

This Review has outlined the changes in cellular and tissular biomechanical properties arising in several pathological processes such as infectious diseases, fibrosis and, most importantly, cancer. We highlighted how immune cells sense these biomechanical changes and tune their response accordingly. The recent advances in the understanding of disease mechanobiology impact on immune surveillance mechanisms gave birth to a promising mechanical immunoengineering field where additional in vivo studies in preclinical models will be instrumental to develop efficient therapies. Preliminary studies have shown that different approaches can leverage immune cell mechano-activation. Nevertheless, each target should be thoroughly analysed and evaluated for its potential for desired therapeutic applications. In the context of cancer in particular, a further and closer inspection of tumour cell mechanics and how it evolves and adapts throughout disease progression is warranted to optimize the inhibitory tools. Although many challenges arise, mechanical modulation of effectors and/or targets, through genetic, pharmacogenetic or nanomedicine approaches is expected to evolve and gain precision in the future. We envision that mechanical immunoengineering will lead to a paradigm shift in the design of next-generation immunotherapy by combining approaches targeting biochemical and biophysical disease–immune interactions.

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V.M., V.G., L.B., W.L., L.T. and J.G.G. researched data for the paper. All authors made substantial contributions to the discussion of content, wrote the paper and reviewed and/or edited the paper before submission.

Competing interests

L.T. is a co-founder, shareholder and advisor for Leman Biotech. The interests of L.T. were reviewed and managed by EPFL. The other authors declare no competing interests.

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