Peer-reviewed version available at Cancers 2018, 10, 199; doi:10.3390/cancers10060199

Review

TGF-β Sustains Tumor Progression Through Biochemical and Mechanical Signal Transduction

Robert L. Furler[1]*, Douglas F. Nixon[1], Christine A. Brantner[2], Anastas Popratiloff[2], and Christel H. Uittenbogaart[3]

[1] Weill Cornell Medicine, Department of Medicine, Division of Infectious Diseases, 413 E 69th St, Belfer Research Building, New York, NY 10021; rlf2001@med.cornell.edu; dnixon@med.cornell.edu
[2] GW Nanofabrication and Imaging Center, Office of the Vice President for Research, George Washington University, Washington, DC 20052, USA.; chrisbrantner@gwu.edu; anastas@gwu.edu
[3] University of California, Los Angeles, Departments of Microbiology, Immunology and Molecular Genetics, Medicine, Pediatrics, UCLA AIDS Institute and the Jonsson Comprehensive Cancer Center, 615 Charles E. Young Drive South, BSRB2, Los Angeles, CA 90095; uittenbo@ucla.edu
* Correspondence: rlf2001@med.cornell.edu

Received: date; Accepted: date; Published: date

Abstract: TGF-β signaling transduces immunosuppressive biochemical and mechanical signals in the tumor microenvironment. In addition to canonical SMAD signaling, TGF-β can promote tumor growth and survival by inhibiting proinflammatory signaling and extracellular matrix remodeling. In this article, we will review how TAK1 activation lies at the intersection of proinflammatory signaling by immune receptors and anti-inflammatory signaling by TGF-β receptors. Additionally, we will discuss the role of TGF-β in the mechanobiology of cancer. Understanding how TGF-β dampens proinflammatory responses and induces pro-survival mechanical signals throughout cancer development will be critical in designing therapeutics which inhibit tumor progression while bolstering the immune response.

Keywords: TGF-β; Cancer; Immunosuppression; TAK1; Mechanobiology; Extracellular Matrix; Tensegrity; DNA damage

1. Introduction

TGF-β signaling can dampen immune responses during cancer progression through biochemical and mechanical signal transduction pathways. Since TGF-β receptors (TGF-βR) are found on several cell types, tumor-derived TGF-β can create a pro-tumorigenic microenvironment by influencing the activity of surrounding leukocytes, endothelial cells, and fibroblasts. The TGF-β Superfamily consists of at least 33 genes (*1*), which are often grouped into either the TGFβ-like family (TGF-β, Activin, Inhibin, Nodal, and Lefty) and the Bone Morphogenetic Protein (BMP)-like family (BMP, GDF, AMH and MIS) (*2*, *3*). Downstream of these receptors, TGF-β can activate SMAD-dependent and SMAD-independent biochemical pathways that promote tumor growth and suppress the immune system (*4*). However, these pathways are not constitutively active. TGF-β is commonly expressed in a latent form is activated following Extracellular Matrix (ECM) remodeling. Subsequent TGF-β signaling increases production of new ECM components. This homeostatic feedback loop is critical for cancer growth. The ECM found within the tumor microenvironment shapes cancer mechanobiology by providing growth signals to the tumor cell and simultaneously suppress immune response.

Despite its well-known immunosuppressive capabilities, TGF-β signaling has been shown to have contrary effects on tumor growth during disease progression (*5-7*). TGF-β family members display anti-tumorigenic and pro-tumorigenic properties depending on the stage of tumor progression (*8-11*).

Early in disease progression, TGF-β appears to play an anti-tumorigenic role by hindering tumor proliferation and metastasis. For example, in early stages of breast cancer, the TGF-β family member BMP7 represses human telomerase reverse transcriptase (hTERT) through a BMPRII and Smad3-dependent manner. Chronic exposure of cancer cells to BMP7 has been shown to induce shortening of cancer cell telomeres and subsequent apoptosis (*12*). TGF-β members can also act on surrounding cells like cancer associated fibroblasts to inhibit tumor progression and metastasis at early stages of disease (*13*).

In contrast, TGF-β signaling takes on a pro-tumorigenic response in later stages of disease. Elevated levels of TGF-β1 in advanced stage breast cancers were associated with tumor size, decreased tumor cell differentiation, epithelial to mesenchymal transition (EMT), and increased metastasis to axillary lymph nodes (*14-18*). EMT and more aggressive phenotypes of late stage prostate cancers were also associated with elevated TGF-β1 (*19*). Inhibiting TGF-β1 receptors or their downstream SMAD signaling at later stages of cancer enhanced chemotherapeutic action (*20-22*) and radiation treatment effects (*23, 24*).

To understand the multifaceted roles of TGF-β in cancer, we will review two ways TGFβ family members promote tumor growth:

- ***TGF-β inhibits proinflammatory signaling in tumor infiltrating leukocytes.***
  The immunosuppressive capabilities of TGF-β have been extensively studied as an external pressure in the cancer setting. TGF-β can dampen pro-inflammatory signals within infiltrating leukocytes via downstream transcription factors called SMADs or through SMAD-independent pathways (*4*). One SMAD-independent pathway that inhibits inflammation is the TGF-β-mediated modulation of TRAF and TAK1 signaling, which lies at an intersection with proinflammatory signaling downstream of IL-1R, TNFR, TLRs, as well as the T cell and B cell receptors.

- ***TGF-β signaling promotes tumor growth & inhibits inflammation through mechanobiology.***
  The ECM provides mechanical cues to surrounding cells through mechanotransduction. ECM remodeling throughout cancer progression is critical for tumor growth, metastasis and angiogenesis; however, extensive ECM degradation can promote inflammation and inhibit proliferation. Cancer cells alter their extracellular environment with proteases during metastasis and build collagen-rich microenvironments at new loci of proliferation. TGF-β signaling plays a large role in the ECM and fibrosis seen within the tumor microenvironment. These ECM proteins can provide pro-tumor growth signals at the same time as providing a physical barrier to infiltrating leukocytes.

TGF-β inhibits proinflammatory signaling cascades and plays a major role in cancer mechanobiology. Both of these effects promote regulatory and anergic immune responses that support tumor survival. Understanding these two mechanisms of TGF-β signaling in tumors will be instrumental in improving cancer immunotherapies.

2. TGF-β Inhibits Proinflammatory Signaling in Tumor Infiltrating Leukocytes

Tumor infiltrating leukocytes can both express and respond to TGF-β. Signaling through TGF-βR can inhibit leukocyte proliferation, differentiation, and survival (*1, 25-28*). These effects can be reversed in leukocytes like macrophages and T cells following inhibition of TGF-β signaling (*29, 30*). Macrophages and T cells (Figure 1) can both produce and respond to TGF-β in the tumor microenvironment.

Tumor associated macrophages often exhibit an immunosuppressive M2 phenotype by expressing IL-10, arginase-1, and TGF-β1 (*31*). TGF-β1 can further inhibit expression of the proinflammatory genes inducible nitric-oxide synthase (INOS) and matrix metalloproteinase-12 (MMP-12) in these macrophages (*32*). Macrophage-derived TGF-β was also shown to enhance Epithelial to

Mesenchymal Transition (EMT) in hepatocellular carcinoma (*33*) and metastasis in non-small cell lung cancer (*34*).

In addition to macrophages, T cells also exhibit immunosuppressive phenotypes in cancer. Despite the presence of tumor antigen-specific T cells in the tumor microenvironment, these cells usually express markers of anergy and senescence (*35, 36*). Elevated TGF-β1 levels were shown to be associated with increased CD4+CD25+FoxP3+ regulatory T cells (Treg), EMT, and more aggressive phenotypes in prostate cancers (*19, 37*). TGF-β1 also induces Treg, NKT cells, and Tr1 cells (*37-41*) and inhibits pro-inflammatory signaling in T cells (*42*). TGF-β can inhibit several proinflammatory signaling cascades in these leukocytes, but we will focus on the role of TGF-β in inhibiting pro-inflammatory biochemical pathways that induce TAK1 activation.

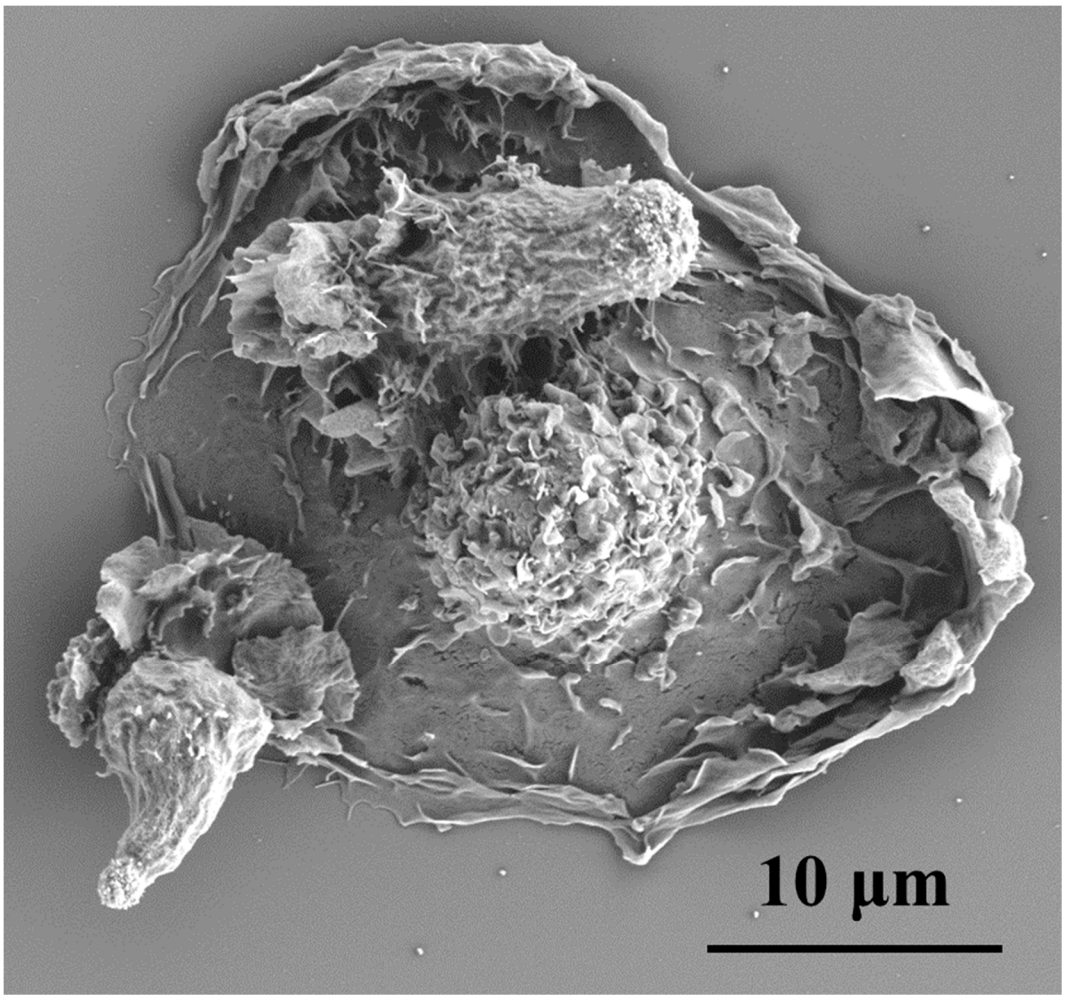

Figure 1. T cells and macrophages exhibit immunosuppressive qualities in tumor microenvironments. Despite presence of macrophages (larger egg-like cell in Scanning Electron Microsopy image taken by our group) and T cells (two smaller cells scanning the surface of the macrophage), TGF-β1 in the tumor microenvironment inhibits proinflammatory signaling in these leukocytes.

As a tumor grows and metastasizes, there is significant tissue damage which causes the release of proinflammatory cytokines that recruit leukocytes. Proinflammatory cytokines like Interleukin-1β (IL-1β) and Tumor Necrosis Factor α (TNFα) signal through transmembrane receptors to alert surrounding cells of homeostatic stress. Along with the IL-1 receptor (IL1R) and TNF receptors (TNFR), proinflammatory signals can be transmitted to leukocytes through Toll-Like Receptors (TLR) and antigen specific receptors on T cells (TCR) and B cells (BCR).

These diverse receptors use TRAF mediators to converge on the activation of TGF-β Activated Kinase-1 (TAK1) to stimulate the NFκB and JNK/p38 MAPK pathways (*43-45*). The JNK and p38 stress pathways induce effectors that eliminate the extracellular stress however prolonged activation of these pathways can induce apoptosis (*44*). The concomitant NFκB activation maintains anti-apoptotic signals until the stress is resolved. TGF-β1, and other TGF-β family members like BMP, also influence TRAF signaling to alter the activity of TAK1 and downstream NFkB/JNK/p38 signaling (*46-48*). Moreover, signals emanating from proinflammatory receptors, including TLR, IL-1R, TNFR, TCR, and BCR can be blunted by TGFβR-mediated alterations of the TRAF/TAK1 signaling axis (Figure 2).

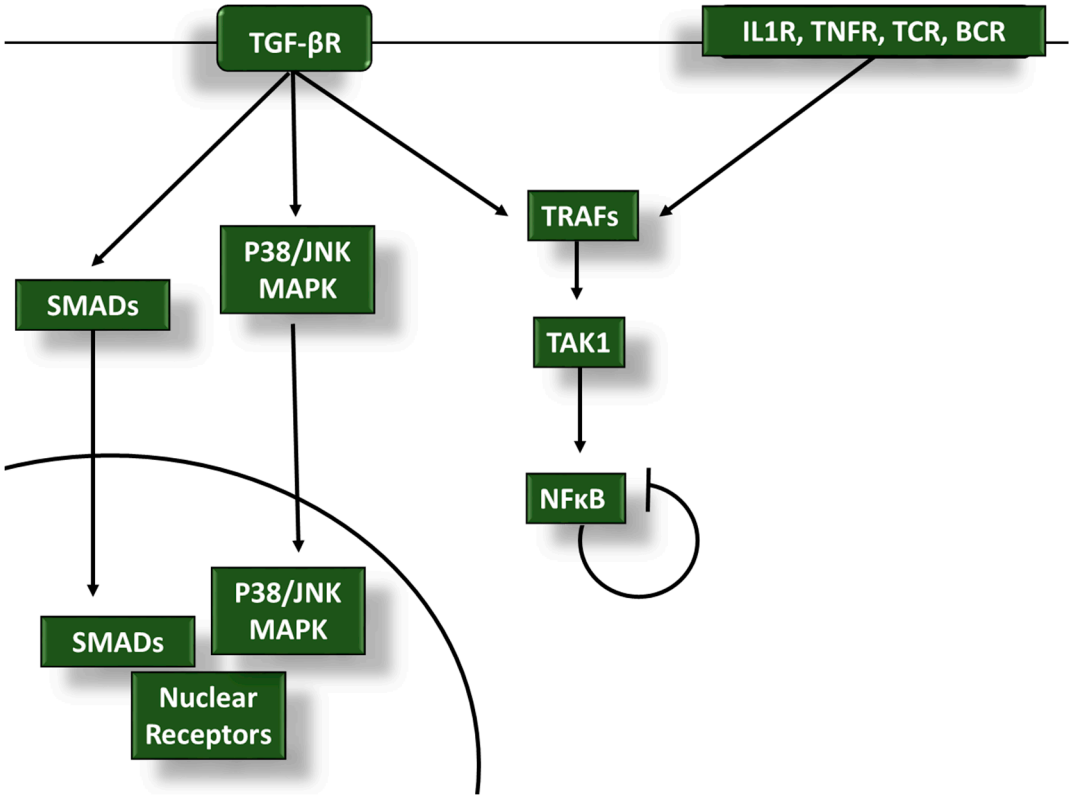

Figure 2. TGF-β Dampens Anti-Tumor Proinflammatory Signaling in Infiltrating Leukocytes Through TAK1, NFκB, and MAP Kinase Modulation: TGF-β signaling interferes with these signaling pathways in tumor associated leukocytes to blunt immune responses during cancer progression.

In addition to inhibiting biochemical proinflammatory signaling cascades within leukocytes, TGF-β can inhibit the immune system and support tumor growth through mechanical transduction pathways.

3. TGF-β Signaling Promotes Tumor Growth and Inhibits Inflammation through Mechanobiology

Mechanobiology links TGF-β, cancer cell survival, and inflammation (*49*). In addition to chemical cues from the environment, cells receive critical homeostatic information from surrounding ECM and neighboring cells through forces applied to adhesion receptors. Remodeling of the ECM throughout cancer progression influences TGF-β signaling and alters these mechanical forces. ECM-derived forces directly impact tumor growth, metastasis, and immune evasion (*50*). In mechanobiology there is a bidirectional information flow between cells and their extracellular environment, called dynamic reciprocity. This relationship between cells and their extracellular environment dictates cellular proliferation, migration, differentiation, and survival (*51-56*). Mechanical information can be

transduced by forces applied to physical linkages between the extracellular environment and the DNA. This mechanical conduit consists of:

- Extracellular Matrix
- Cell Adhesion Receptors
- Cytoskeleton
- Nuclear Membrane Adaptors
- Chromatin

Many eukaryotic cells require adhesions and these other mechanical components to stimulate growth and survival. Typical cells also inhibit their proliferation in densely packed environments. However, cancer cells exhibit anchorage independence and lack density-dependent growth inhibition. The ability of tumors to overcome these mechanical checkpoints may be due to elevated levels of TGF-β signaling that strengthens dynamic reciprocity in the tumor microenvironment. We will review how TGF-β signaling influences ECM structure and nuclear mechanobiology in cancer.

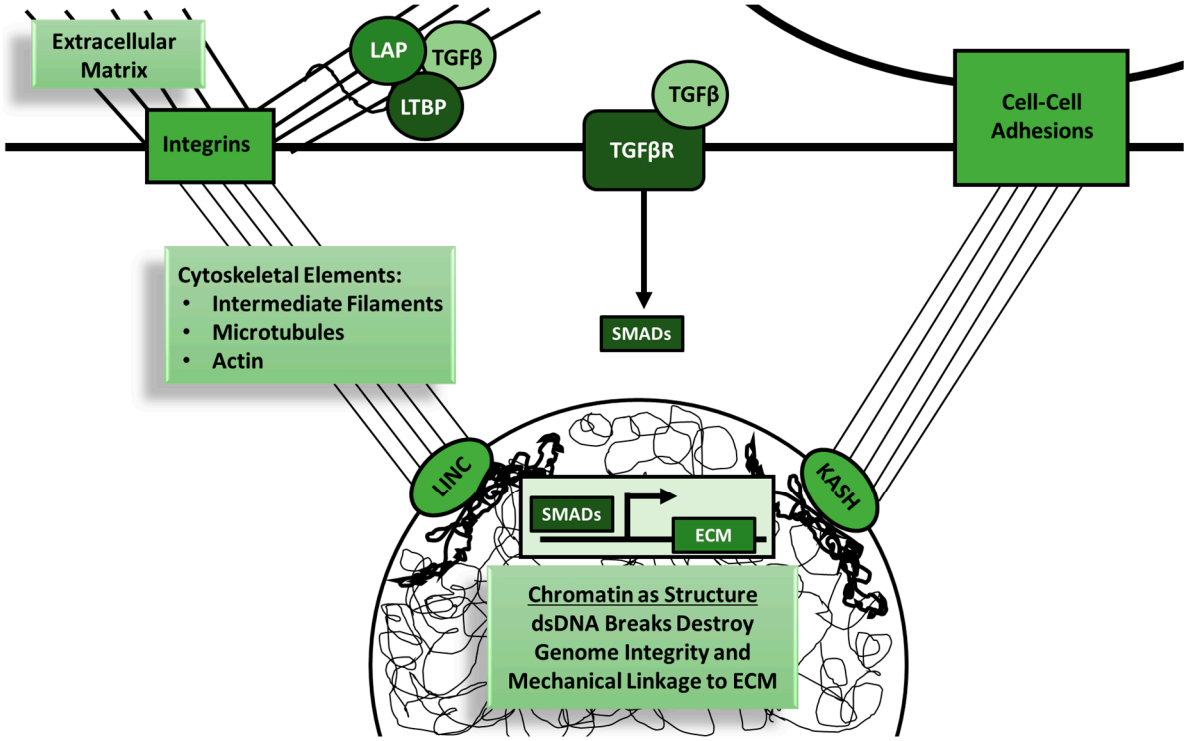

Figure 3. TGF-β signaling is intimately linked with the mechanobiology of cells. Dynamic reciprocity is the concept of bidirectional influence of cells and their microenvironment, including adhesions to the extracellular matrix and to surrounding cells. Physical linkages between the extracellular microenvironment are created by plasma membrane adhesion receptors, the cytoskeleton, nuclear membrane KASH-domain proteins, and chromatin. TGF-β signaling plays an important role in this dynamic reciprocity at both the extracellular matrix and nuclear levels. Extracellular matrix degradation or remodeling relieves mechanical tension on the cell down to the nuclear level while simultaneously increasing the bioavailability of active TGF-β. DNA damage can also decrease structural integrity of this mechanical tension. TGF-β signaling restores this mechanical homeostasis through upregulation of extracellular matrix components and DNA repair enzymes. Tumor cells with elevated TGF-β signaling are able to restore mechanical homeostasis despite ongoing ECM remodeling and DNA damage.

3.1. TGF-β & Extracellular Matrix

Dynamic reciprocity is exemplified by TGF-β activation and ECM homeostasis (Figure 3). TGF-β proteins are produced by several cell types and secreted in an inactive form along with latency-associated peptide (LAP), which associate with latent TGF-β-binding proteins (LTBPs) (*57*, *58*). These latent TGF-β complexes bind to ECM proteins including fibrillins and fibronectins. TGF-β is

activated when LAP or the ECM is degraded during cancer, infection, or wounding (*59*). Following a mechanical cue that the ECM has been degraded, activated TGF-β family members (including TGF-β1, BMP, Activins, and Growth Differentiation Factors) signal through TGF-βR to induce expression of new ECM proteins (*60, 61*). After newly expressed matrix proteins are secreted, the remaining TGF-β return to its latent state. In addition to biochemical signals derived from TGF-βR signaling, ECM degradation transmits mechanical signals to surrounding cells by releasing tension on cell adhesions. These mechanical signals are influenced by TGF-β signaling and can change nuclear shape and gene expression in tumor cells.

Cancers use dynamic reciprocity to grow, metastasize, and evade the immune system by remodeling the ECM at different stages of disease progression. The ECM consists of proteins, glycoproteins, glycosaminoglycans, and other molecules that function as adhesive substrates that promote signaling through integrins, growth factors and mechanical cues (Figure 4).

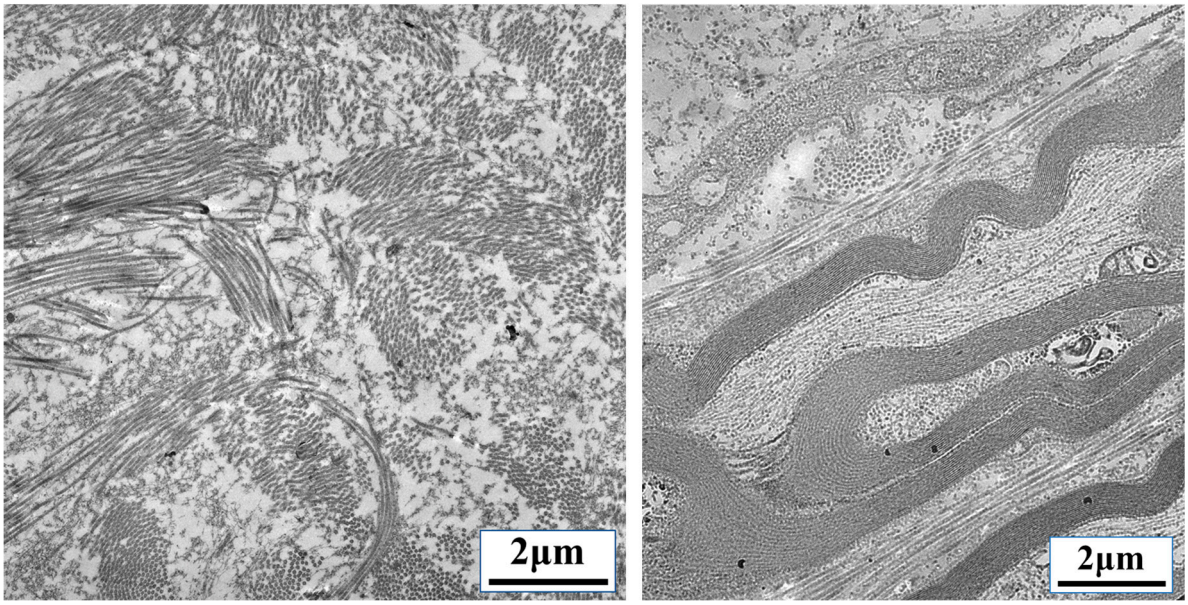

Figure 4. Extracellular matrix interactions provide mechanical cues to infiltrating cells. Extracellular matrix remodeling is required for tumor proliferation, metastasis, angiogenesis, and leukocyte infiltration. The image on the left shows a region with multidirectional bundles of collagen. The image on the right shows highly organized bundles of parallel collagen fibers found in body. Dense collagen networks like these found in capsular regions of lymphoid tissue can be found within the tumor microenvironment. Collagen induced by TGF-β and other factors provide structural support to cancer cells and a physical barrier to leukocytes. (TEM images taken by our group)

During metastasis and angiogenesis, the ECM is degraded by tumor-derive proteases, which stimulates the release and activation of growth factors (*62*) and increases the bioactivity of latent TGF-β1 (*63*). Following metastasis to a secondary site, the tumors use this bioactive TGF-β to induce expression of ECM components including collagen, fibronectin, and tenascin C along with ECM-related enzymes and chaperone proteins (*64, 65*). Tumor associated macrophages also produce TGF-β that signals fibroblasts produce collagen and other ECM proteins (*31, 33*). This ECM remodeling creates a microenvironment amenable to tumor proliferation and immunosuppression.

The excessive buildup of ECM proteins is called fibrosis and contributes to the progressive ECM rigidity seen during cancer progression and throughout other chronic diseases (*66-68*). In a breast cancer model, Liverani *et.al.* showed that a more aggressive tumor cell line MDA-MB-231 induced higher collagen content, collagen crosslinking, and increased ECM stiffness compared to the less aggressive cell line MCF-7 (*69*). Progressive stiffening and reorganization of the ECM during cancer

progression is also due to TGF-β-mediated effects on surrounding stromal cells including fibroblasts. Reactive stromal cells in human prostate cancers have a myofibroblast phenotype that exhibit increased collagen I production, vimentin, tenascin, and smooth muscle α-actin. Elevated TGF-β1 levels were also found in these prostatic intra-epithelial neoplasia (*70*). TGF-β1 induces contraction of stromal fibroblasts and subsequent ECM strain (*71*). This progressive ECM stiffness is directly correlated with tumor aggressiveness and can dampen the immune response (*50, 72-75*).

Tissue fibrosis has several detrimental effects during chronic diseases. In addition to local disturbances in tissue function, fibrosis creates a physical barrier to infiltrating immune effectors and potential therapeutics agents. Netti *et.al.* reported that fibrotic ECM prevents therapeutic IgG from penetrating solid tumors (*76*) and others have shown that chemotherapeutic drugs may not reach fibrotic tissue in small cell lung cancers (*77*).

3.2. TGF-β & Nuclear Mechanobiology in Cancer

Tensegrity is a structural concept that can be used to understand the mechanics of 3-dimensional structures like cells. Cellular structures are physically connected through cytoskeletal elements and forces applied to one area can have effects throughout the cell (*52-56, 78-82*). Forces from the ECM in the tumor microenvironment can have direct impacts on gene expression in tumor and immune cells. These mechanical forces are transmitted through cellular adhesions containing receptors like integrins, which are one of the primary transducers of mechanical information from the environment to the nucleus (*78, 80, 82, 83*). TGF-β can be activated by and alter expression of integrins during cancer progression (*84-88*). Integrins and other cell adhesions transduce extracellular forces mechanically to the nucleus via the cytoskeleton (Figure 5). Cytoskeletal elements like actin, microtubules, and vimentin have all been shown to play a role in TGF-β signaling and cancer (*89, 90*). Tumor metastasis and proliferation are both altered by TGF-β-mediated cytoskeletal changes (*91, 92*). The cytoskeleton attaches to the nuclear lamina and chromatin through nuclear membrane adaptor proteins called LInker of Nucleoskeleton and Cytoskeleton (LINC) complexes and KASH domain proteins (*93*). TGF-β signaling and some nuclear lamina proteins have been shown to have reciprocal regulation (*94-96*). Forces emanating from the extracellular environment are transduced through these structural proteins and can alter 3-dimensional nuclear shape and directly impact gene expression (*52, 53, 55, 79-81, 97-99*). TGF-β can influence the structural integrity of each of these sections of the mechanical conduit from the ECM to the nucleus. Additionally, TGF-β influences the structural integrity of chromatin and DNA.

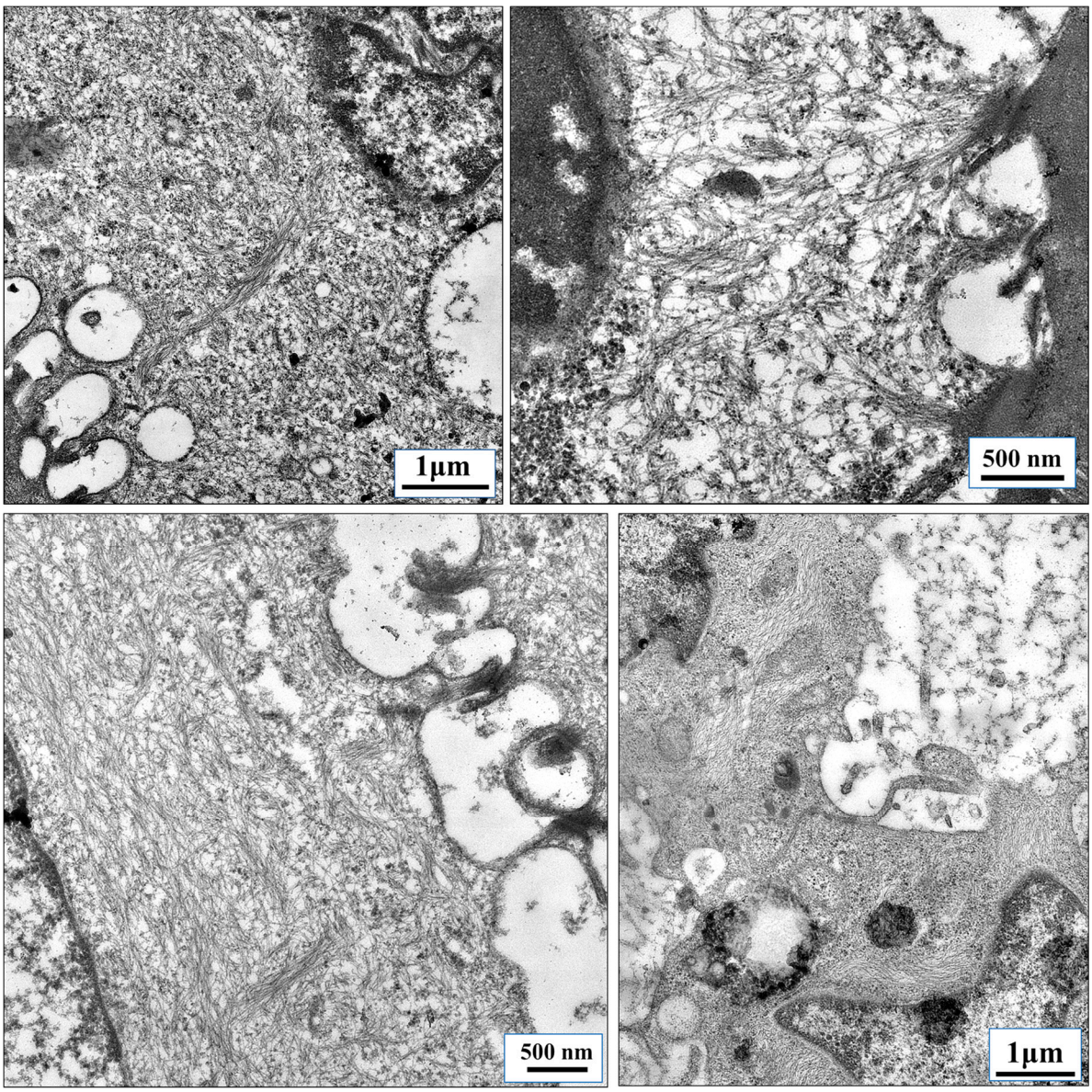

Figure 5. Cell adhesions to other cells and the extracellular matrix are mechanically linked to the nucleus. Leukocytes and metastatic tumors often migrate to secondary lymphoid tissues. As seen from the lymphoid tissue images above, cells are intimately connected through several cell:cell adhesions as they migrate and proliferate in this environment. The four images above show that cell adhesions are directly linked to the nuclear envelope by cytoskeletal elements (Transmission Electron Microscopy images taken by our group).

The role of mechanical forces in gene expression and the physical linkages between the cell adhesions and the chromatin (Figure 6) lead to the idea of chromatin as structural information. With the development of new technologies like Hi-C, more emphasis is being placed on the importance of genome structure (*100, 101*). Genome architecture and chromosome domains are recapitulated through chromatin cross-linking following by DNA sequencing. These approaches give a snapshot of how the genome is organized at a specific timepoint. Genomic architecture changes with time and cellular differentiation, but can be influenced by TGF-β and DNA damage. The integrity of the genome is also critical to maintain, exemplified by the quick repair response that is initiated following DNA damage, as prolonged DNA damage typically leads to apoptosis. Maintenance of DNA structural integrity may be critical for the dynamic reciprocity between the cell and its environment through tensegrity.

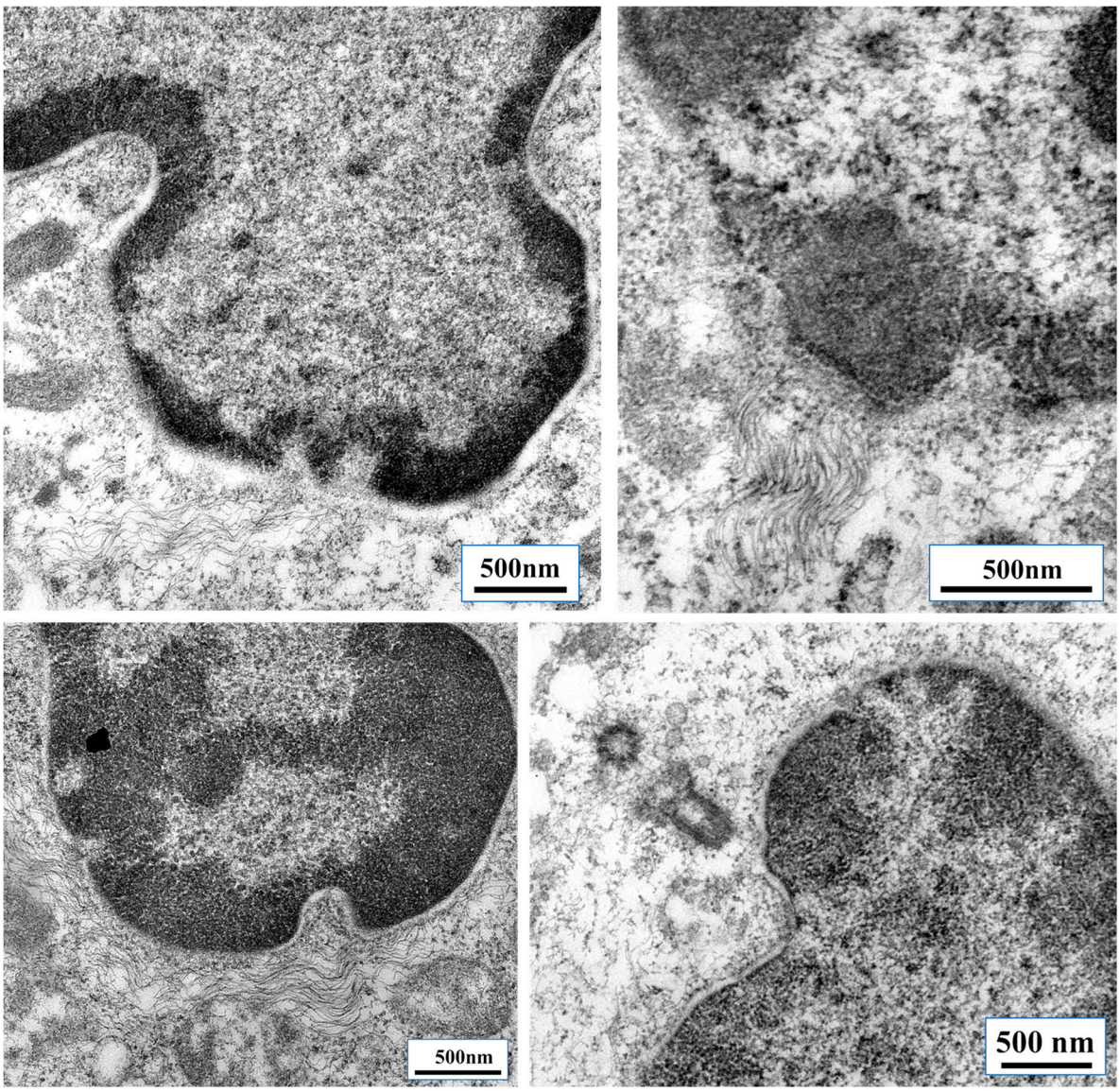

Figure 6. The Cytoskeleton Can Alter Nuclear Shape. Cell adhesions are directly linked to the nuclear lamina and chromatin via the cytoskeleton and LINC complexes. Forces applied to cellular adhesions have been shown to change nuclear shape and alter gene expression. Deformations at the site of cytoskeletal attachment to the nucleus can be seen in the four images above. As shown in these leukocytes, the fibrillar cytoskeletal elements are connected directly to the nucleus, primarily at sites of darkened areas of heterochromatin. Microtubules are organized at the centrosome, which contains centrioles. Microtubules are one type of cytoskeletal protein that links adhesion receptors to the nucleus. The bottom right image shows a pair of centrioles and their close proximity to the nuclear envelope. (TEM images taken by our group)

Cancer cells are continuously exposed to DNA damage, yet they persist with the help of TGF-β signaling. TGF-β signaling modulates the DNA repair response and apoptosis in normal and cancerous cells. Some reports have shown that TGF-β and SMAD signaling enhance the DNA damage response to maintain genomic stability (*102, 103*); however, others have shown that CD44+/CD24- cancer cells with constitutively activated TGF-β signaling are defective in DNA-repair that make their genomes less clonal following dsDNA breaks (*104*). The SMADs have been shown to interact with p53 and also regulate transcription of other DNA repair enzymes (*105*). Other reports indicate that TGF-β signaling aids in tumor growth by inhibiting DNA damage-induced apoptosis. Elevated TGF-β1 signaling has been shown to confer radioresitance following double-stranded DNA damage caused by ionizing radiation (*106*). TGF-β1/SMAD signaling inhibits p53 activation at both transcriptional and translational levels and subsequent apoptosis in precancerous

cells (*107*). Therefore, inhibition of TGF-β1 signaling has been recommended as a possible avenue to promote cancer cell death following genotoxic stress after radiation treatment (*23, 24*).

In addition to DNA damage and genome integrity, the structure of the nucleus changes with tumor behavior. ECM remodeling during cancer progression has been associated with nuclear deformations, subsequent gene activation, and EMT (*108*). These nuclear structural changes can occur due to forces generated during ECM remodeling. During the early stages of cancer, the surrounding ECM can be a hinderance, as dense fibrillar ECM obstructs tumor growth, metastasis, and angiogenesis. Cancers need to degrade surrounding collagen by matrix metalloproteinases and other enzymes to overcome this physical hurdle (*109*). In addition to collagen, disturbances in hyalectan proteoglycans during cancer progression has been seen along with the activation of A Disintegrin-like And Metalloproteinase domain with Thrombospondin-1 motif (ADAMTS) enzymes (*110*). The degraded matrix releases tension the tumor cell, which can sense this mechanical signal and alter its gene expression. This mechanical stimulus may be an influential step in Epithelial to Mesenchymal Transition (EMT).

ECM stiffening during cancer progression is associated with matrix crosslinking or reorganization. In the tumor microenvironment, ECM fibrils become organized in parallel directions. These fibrils are referred to as 'tumor-associated collagen signatures' (TACS) and have been suggested to create migration routes for metastasizing cancer cells (*111*) like those used by leukocytes (Figure 7). Migration through tissues with dense ECM requires nuclear deformation and extension. This is seen both in leukocytes and cancer cells and is one more example of how nuclear structure can be altered. The mechanical forces placed on the nucleus during migration may be critical for gene expression and cellular differentiation not only in tumor cells and leukocytes, but for all developing cells.

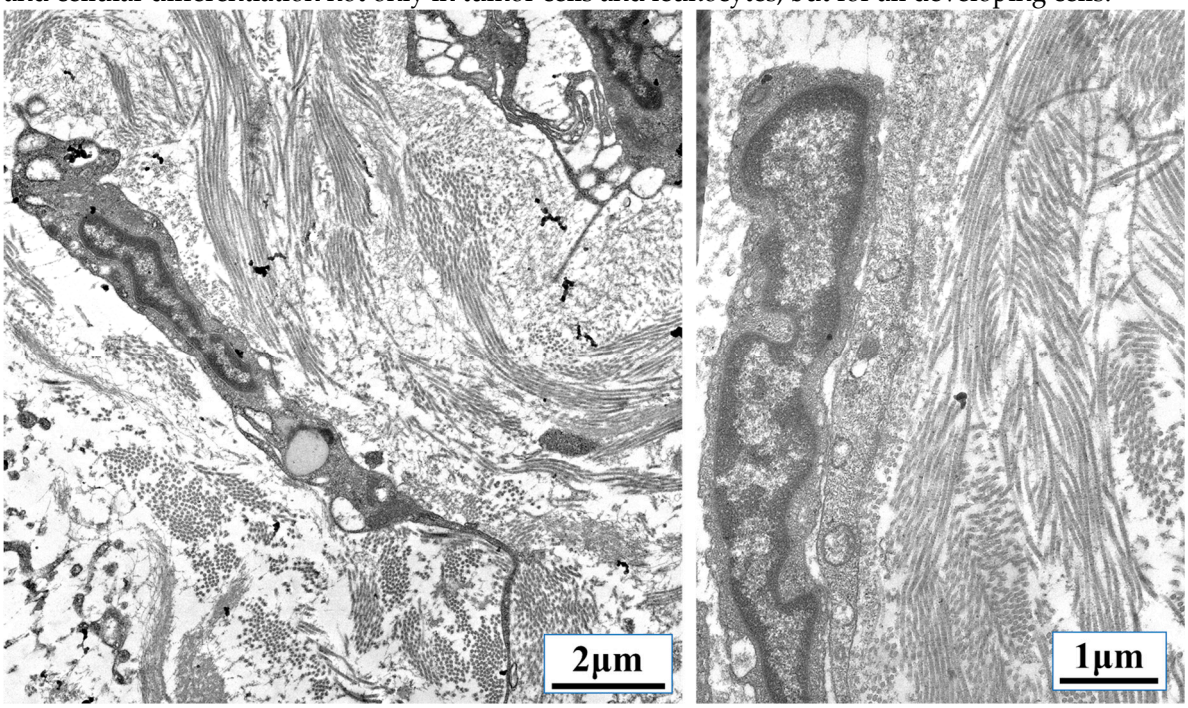

Figure 7. Parallel organization of ECM fibrils can be used as migration tracts. TGF-β induced fibrosis and ECM remodeling during cancer transmits mechanical information from the tumor microenvironment directly to the nucleus of tumor cells, infiltrating leukocytes, fibroblasts, and endothelial cells. Some metastasizing cancer cells used parallel ECM fibrils called 'tumor-associated collagen signatures' (TACS), similar to the tracts used by the leukocytes shown above migrating through lymphoid tissue. (TEM images taken by our group)

4. Materials and Methods

Scanning electron microscopy (SEM) was done on peripheral blood lymphocytes grown in RPMI growth medium supplemented with 10% Fetal Bovine Serum (FBS), 20IU/mL IL-2, and antibiotics. The cells were plated on fibronectin-coated coverslips for 3-7 days. Adherent cells were fixed with 2.5% glutaraldehyde, 1% paraformaldehyde and 0.12M sodium cacodylate buffer (pH 7.2-7.4) for 20 minutes at room temperature followed by 40 minutes shaking on ice. Following rinsing, the cells were stained with 1% osmium tetroxide (Electron Microscopy Sciences) for 60 minutes on ice. The cells were dehydrated through a series of ethyl alcohol/deionized water solutions followed by critical point drying and sputter coating with iridium. Imaging was done using a FEI Teneo LV SEM instrument (Thermo Fisher Scientific).

Transmission electron microscopy (TEM) was done on human lymphoid tissue. Routine EM processing was done using intact human tonsillar tissue. The tissue was fixed 2.5% glutaraldehyde, 1% paraformaldehyde in 0.12 M sodium cacodylate buffer for 20 minutes at room temperature followed by 40 minutes shaking on ice. The tissue was mechanically sectioned into ~1 mm^3 tissue chunks. Cells were then stained for one hour in 1% osmium tetroxide (Electron Microscopy Sciences). The cells were dehydrated through a series of ethyl alcohol/deionized water solutions and propylene oxide before embedding in LR White resin. Blocks were cured for 48 h at 60 °C. Polymerized blocks were trimmed and 70 nm ultrathin sections were cut with a diamond knife on a Leica Ultramicut EM UC7 before transferring them to 200 mesh copper grids. Sections were counterstained with 1% uranyl acetate for 10 minutes and lead citrate for 2 minutes. Samples were imaged with a FEI Talos F200X-TEM (Thermo Fisher Scientific) operating at an accelerating voltage of 80 kV equipped with a Ceta™ 4K x 4K camera.

5. Conclusions

The multifunctional cytokine TGF-β has been shown to have several pro-tumorigenic actions during cancer progression. TGF-β signaling can mediate immune suppression through SMAD-dependent and SMAD-independent pathways. TGF-β signaling can antagonize proinflammatory signals emanating from immune receptors (IL-1R, TLR, TNFR, TCR, and BCR) by modulating TRAF/TAK1, NFκB and p38/JNK MAP Kinase activation. In addition to classical biochemical signaling cascades, TGF-β in the tumor microenvironment activates pro-tumor and anti-inflammatory mechanotransduction pathways through extracellular matrix remodeling, cytoskeletal alterations, and maintenance of DNA damage. Understanding how TGF-β signaling affects proinflammatory signaling and cancer mechanobiology will be critical in designing therapeutics which inhibit tumor progression while bolstering the immune response (*112*).

Acknowledgments: The authors wish to thank Dr. Thomas Troost for supplying tissue samples for these studies. This publication resulted in part from research supported by the District of Columbia Center for AIDS Research, an NIH funded program (AI117970), which is supported by the following NIH Co-Funding and Participating Institutes and Centers: NIAID, NCI, NICHD, NHLBI, NIDA, NIMH, NIA, FIC, NIGMS, NIDDK, and OAR. This work was also supported by the UCLA AIDS Institute, UCLA CFAR NIH AI-28697. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Author Contributions: R.L.F, D.F.N, and C.H.U conceived and designed the experiments; R.L.F, C.A.B., and A.P. performed the experiments; R.L.F., D.F.N, C.A.B., A.P. and C.H.U. analyzed the data; D.F.N., C.A.B., and A.P. contributed reagents/materials/analysis tools; R.L.F and C.H.U.wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. W. Chen, P. Ten Dijke, Immunoregulation by members of the TGFbeta superfamily. *Nat Rev Immunol* **16**, 723 (Nov 25, 2016).
2. A. Weiss, L. Attisano, The TGFbeta superfamily signaling pathway. *Wiley Interdiscip Rev Dev Biol* **2**, 47 (Jan-Feb, 2013).

3. M. de Caestecker, The transforming growth factor-beta superfamily of receptors. *Cytokine Growth Factor Rev* **15**, 1 (Feb, 2004).
4. R. Derynck, Y. E. Zhang, Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* **425**, 577 (Oct 9, 2003).
5. B. Tang *et al.*, TGF-beta switches from tumor suppressor to prometastatic factor in a model of breast cancer progression. *The Journal of clinical investigation* **112**, 1116 (Oct, 2003).
6. G. Wilding, G. Zugmeier, C. Knabbe, K. Flanders, E. Gelmann, Differential effects of transforming growth factor beta on human prostate cancer cells in vitro. *Molecular and cellular endocrinology* **62**, 79 (Mar, 1989).
7. C. J. David *et al.*, TGF-beta Tumor Suppression through a Lethal EMT. *Cell* **164**, 1015 (Feb 25, 2016).
8. K. Park *et al.*, Genetic changes in the transforming growth factor beta (TGF-beta) type II receptor gene in human gastric cancer cells: correlation with sensitivity to growth inhibition by TGF-beta. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 8772 (Sep 13, 1994).
9. Y. Tang *et al.*, Progenitor/stem cells give rise to liver cancer due to aberrant TGF-beta and IL-6 signaling. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 2445 (Feb 19, 2008).
10. J. J. Yin *et al.*, TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. *The Journal of clinical investigation* **103**, 197 (Jan, 1999).
11. J. Massague, TGFbeta in Cancer. *Cell* **134**, 215 (Jul 25, 2008).
12. L. Cassar *et al.*, TGF-beta receptor mediated telomerase inhibition, telomere shortening and breast cancer cell senescence. *Protein Cell* **8**, 39 (Jan, 2017).
13. S. Busch *et al.*, TGF-beta receptor type-2 expression in cancer-associated fibroblasts regulates breast cancer cell growth and survival and is a prognostic marker in pre-menopausal breast cancer. *Oncogene* **34**, 27 (Jan 2, 2015).
14. J. M. Zarzynska, Two faces of TGF-beta1 in breast cancer. *Mediators Inflamm* **2014**, 141747 (2014).
15. S. H. Mahdi, H. Cheng, J. Li, R. Feng, The effect of TGF-beta-induced epithelial-mesenchymal transition on the expression of intracellular calcium-handling proteins in T47D and MCF-7 human breast cancer cells. *Arch Biochem Biophys* **583**, 18 (Oct 1, 2015).
16. S. D. Boone *et al.*, Associations between genetic variants in the TGF-beta signaling pathway and breast cancer risk among Hispanic and non-Hispanic white women. *Breast Cancer Research and Treatment* **141**, 287 (Sep, 2013).
17. C. Xu *et al.*, Co-expression of parathyroid hormone related protein and TGF-beta in breast cancer predicts poor survival outcome. *BMC Cancer* **15**, 925 (Nov 23, 2015).
18. A. Calon *et al.*, Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. *Cancer cell* **22**, 571 (Nov 13, 2012).
19. C. T. Wu, Y. H. Chang, W. Y. Lin, W. C. Chen, M. F. Chen, TGF Beta1 Expression Correlates with Survival and Tumor Aggressiveness of Prostate Cancer. *Ann Surg Oncol* **22 Suppl 3**, S1587 (Dec, 2015).
20. N. E. Bhola *et al.*, TGF-beta inhibition enhances chemotherapy action against triple-negative breast cancer. *Journal of Clinical Investigation* **123**, 1348 (Mar, 2013).
21. S. H. Wrzesinski, Y. Y. Wan, R. A. Flavell, Transforming growth factor-beta and the immune response: implications for anticancer therapy. *Clin Cancer Res* **13**, 5262 (Sep 15, 2007).
22. K. E. de Visser, W. M. Kast, Effects of TGF-beta on the immune system: implications for cancer immunotherapy. *Leukemia* **13**, 1188 (Aug, 1999).

23. J. Kirshner *et al.*, Inhibition of transforming growth factor-beta 1 signaling attenuates ataxia telanglectasia mutated activity in response to genotoxic stress. *Cancer research* **66**, 10861 (Nov 15, 2006).
24. S. Du *et al.*, Attenuation of the DNA damage response by transforming growth factor-beta inhibitors enhances radiation sensitivity of non-small-cell lung cancer cells in vitro and in vivo. *International journal of radiation oncology, biology, physics* **91**, 91 (Jan 1, 2015).
25. B. A. Teicher, Transforming growth factor-beta and the immune response to malignant disease. *Clin Cancer Res* **13**, 6247 (Nov 1, 2007).
26. R. L. Furler, C. H. Uittenbogaart, GLI2 regulates TGF-beta1 in human CD4+ T cells: implications in cancer and HIV pathogenesis. *PloS one* **7**, e40874 (2012).
27. G. Strassmann, D. R. Bertolini, O. Eidelman, Inhibition of immune reactions in vivo by liposome associated transforming growth factor (TGF) type beta 1. *Clin Exp Immunol* **86**, 532 (Dec, 1991).
28. S. A. Oh, M. O. Li, TGF-beta: guardian of T cell function. *J Immunol* **191**, 3973 (Oct 15, 2013).
29. L. Yang, Y. Pang, H. L. Moses, TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression. *Trends Immunol* **31**, 220 (Jun, 2010).
30. T. Fujita *et al.*, Inhibition of transforming growth factor-beta-mediated immunosuppression in tumor-draining lymph nodes augments antitumor responses by various immunologic cell types. *Cancer research* **69**, 5142 (Jun 15, 2009).
31. N. B. Hao *et al.*, Macrophages in tumor microenvironments and the progression of tumors. *Clinical & developmental immunology* **2012**, 948098 (2012).
32. F. Werner *et al.*, Transforming growth factor-beta 1 inhibition of macrophage activation is mediated via Smad3. *The Journal of biological chemistry* **275**, 36653 (Nov 24, 2000).
33. Q. M. Fan *et al.*, Tumor-associated macrophages promote cancer stem cell-like properties via transforming growth factor-beta1-induced epithelial-mesenchymal transition in hepatocellular carcinoma. *Cancer letters* **352**, 160 (Oct 1, 2014).
34. S. Zhang *et al.*, Tumor-associated macrophages promote tumor metastasis via the TGF-beta/SOX9 axis in non-small cell lung cancer. *Oncotarget* **8**, 99801 (Nov 21, 2017).
35. J. Crespo, H. Sun, T. H. Welling, Z. Tian, W. Zou, T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. *Curr Opin Immunol* **25**, 214 (Apr, 2013).
36. P. S. Kim, R. Ahmed, Features of responding T cells in cancer and chronic infection. *Curr Opin Immunol* **22**, 223 (Apr, 2010).
37. L. Zhang, H. Yi, X. P. Xia, Y. Zhao, Transforming growth factor-beta: an important role in CD4+CD25+ regulatory T cells and immune tolerance. *Autoimmunity* **39**, 269 (Jun, 2006).
38. Y. Y. Wan, R. A. Flavell, TGF-beta and regulatory T cell in immunity and autoimmunity. *J Clin Immunol* **28**, 647 (Nov, 2008).
39. W. Chen, S. Perruche, J. Li, CD4+CD25+ T regulatory cells and TGF-beta in mucosal immune system: the good and the bad. *Curr Med Chem* **14**, 2245 (2007).
40. A. J. Theron, R. Anderson, T. M. Rossouw, H. C. Steel, The Role of Transforming Growth Factor Beta-1 in the Progression of HIV/AIDS and Development of Non-AIDS-Defining Fibrotic Disorders. *Front Immunol* **8**, 1461 (2017).
41. Y. Y. Wan, R. A. Flavell, Regulatory T cells, transforming growth factor-beta, and immune suppression. *Proceedings of the American Thoracic Society* **4**, 271 (Jul, 2007).
42. M. O. Li, Y. Y. Wan, S. Sanjabi, A. K. L. Robertson, R. A. Flavell, Transforming growth factor-beta regulation of immune responses. *Annual Review of Immunology* **24**, 99 (2006).

43. M. Landstrom, The TAK1-TRAF6 signalling pathway. *Int J Biochem Cell Biol* **42**, 585 (May, 2010).
44. M. Yamashita *et al.*, TRAF6 mediates Smad-independent activation of JNK and p38 by TGF-beta. *Molecular cell* **31**, 918 (Sep 26, 2008).
45. G. Takaesu *et al.*, TAB2, a novel adaptor protein, mediates activation of TAK1 MAPKKK by linking TAK1 to TRAF6 in the IL-1 signal transduction pathway. *Molecular cell* **5**, 649 (Apr, 2000).
46. A. Sorrentino *et al.*, The type I TGF-beta receptor engages TRAF6 to activate TAK1 in a receptor kinase-independent manner. *Nature cell biology* **10**, 1199 (Oct, 2008).
47. H. Shibuya *et al.*, Role of TAK1 and TAB1 in BMP signaling in early Xenopus development. *The EMBO journal* **17**, 1019 (Feb 16, 1998).
48. K. Yamaguchi *et al.*, XIAP, a cellular member of the inhibitor of apoptosis protein family, links the receptors to TAB1-TAK1 in the BMP signaling pathway. *The EMBO journal* **18**, 179 (Jan 4, 1999).
49. V. Vogel, Unraveling the Mechanobiology of Extracellular Matrix. *Annu Rev Physiol* **80**, 353 (Feb 10, 2018).
50. M. A. Swartz, A. W. Lund, Lymphatic and interstitial flow in the tumour microenvironment: linking mechanobiology with immunity. *Nat Rev Cancer* **12**, 210 (Feb 24, 2012).
51. J. D. Humphrey, E. R. Dufresne, M. A. Schwartz, Mechanotransduction and extracellular matrix homeostasis. *Nature reviews. Molecular cell biology* **15**, 802 (Dec, 2014).
52. D. E. Ingber, Mechanical control of tissue morphogenesis during embryological development. *The International journal of developmental biology* **50**, 255 (2006).
53. D. E. Ingber, Tensegrity II. How structural networks influence cellular information processing networks. *Journal of cell science* **116**, 1397 (Apr 15, 2003).
54. D. E. Ingber, Tensegrity I. Cell structure and hierarchical systems biology. *Journal of cell science* **116**, 1157 (Apr 1, 2003).
55. D. E. Ingber, Cellular mechanotransduction: putting all the pieces together again. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **20**, 811 (May, 2006).
56. D. E. Ingber, N. Wang, D. Stamenovic, Tensegrity, cellular biophysics, and the mechanics of living systems. *Rep Prog Phys* **77**, 046603 (Apr, 2014).
57. A. B. Roberts, B. K. McCune, M. B. Sporn, TGF-beta: regulation of extracellular matrix. *Kidney Int* **41**, 557 (Mar, 1992).
58. I. B. Robertson *et al.*, Latent TGF-beta-binding proteins. *Matrix Biol* **47**, 44 (Sep, 2015).
59. R. O. Hynes, The Extracellular Matrix: Not Just Pretty Fibrils. *Science* **326**, 1216 (Nov 27, 2009).
60. J. P. Rys, D. A. Monteiro, T. Alliston, Mechanobiology of TGFbeta signaling in the skeleton. *Matrix Biol* **52-54**, 413 (May-Jul, 2016).
61. M. S. Lim, K. W. Jeong, Role of Flightless-I (Drosophila) homolog in the transcription activation of type I collagen gene mediated by transforming growth factor beta. *Biochemical and biophysical research communications* **454**, 393 (Nov 21, 2014).
62. Y. A. DeClerck *et al.*, Proteases, extracellular matrix, and cancer: a workshop of the path B study section. *Am J Pathol* **164**, 1131 (Apr, 2004).
63. F. Klingberg *et al.*, Prestress in the extracellular matrix sensitizes latent TGF-beta1 for activation. *J Cell Biol* **207**, 283 (Oct 27, 2014).
64. R. X. Gao-Feng Xiong, Function of cancer cell-derived extracellular matrix in tumor progression. *Journal of Cancer Metastasis and Treatment* **2**, 357 (2016).

65. M. M. Geng *et al.*, Use of representational difference analysis to study the effect of TGFB on the expression profile of a pancreatic cancer cell line. *Genes, chromosomes & cancer* **26**, 70 (Sep, 1999).
66. B. Hinz, The extracellular matrix and transforming growth factor-beta1: Tale of a strained relationship. *Matrix Biol* **47**, 54 (Sep, 2015).
67. K. A. Jansen *et al.*, A guide to mechanobiology: Where biology and physics meet. *Biochimica Et Biophysica Acta-Molecular Cell Research* **1853**, 3043 (Nov, 2015).
68. S. Altinay, Is Extracellular Matrix a Castle Against to Invasion of Cancer Cells? *Tumor Metastasis*, 23 (2016).
69. L. M. Chiara Liverani, Luca Cristofolini, Emanuele Giordano, Silvia Minardi, Giovanna Della Porta, Alessandro De Vita, Giacomo Miserocchi, Chiara Spadazzi, Ennio Tasciotti, Dino Amadori, and Toni Ibrahim, Investigating the Mechanobiology of Cancer Cell-ECM Interaction Through Collagen-Based 3D Scaffolds. *Cellular and Molecular Bioengineering* **10**, 223 (June 2017, 2017).
70. J. A. Tuxhorn *et al.*, Reactive stroma in human prostate cancer: induction of myofibroblast phenotype and extracellular matrix remodeling. *Clin Cancer Res* **8**, 2912 (Sep, 2002).
71. B. H. Campbell, C. Agarwal, J. H. Wang, TGF-beta1, TGF-beta3, and PGE(2) regulate contraction of human patellar tendon fibroblasts. *Biomech Model Mechanobiol* **2**, 239 (Jun, 2004).
72. T. R. Cox, J. T. Erler, Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. *Dis Model Mech* **4**, 165 (Mar, 2011).
73. S. P. Carey, T. M. D'Alfonso, S. J. Shin, C. A. Reinhart-King, Mechanobiology of tumor invasion: engineering meets oncology. *Crit Rev Oncol Hematol* **83**, 170 (Aug, 2012).
74. R. Jinka, R. Kapoor, P. G. Sistla, T. A. Raj, G. Pande, Alterations in Cell-Extracellular Matrix Interactions during Progression of Cancers. *International journal of cell biology* **2012**, 219196 (2012).
75. P. Lu, V. M. Weaver, Z. Werb, The extracellular matrix: a dynamic niche in cancer progression. *J Cell Biol* **196**, 395 (Feb 20, 2012).
76. P. A. Netti, D. A. Berk, M. A. Swartz, A. J. Grodzinsky, R. K. Jain, Role of extracellular matrix assembly in interstitial transport in solid tumors. *Cancer research* **60**, 2497 (May 1, 2000).
77. T. Sethi *et al.*, Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: A mechanism for small cell lung cancer growth and drug resistance in vivo. *Nature Medicine* **5**, 662 (Jun, 1999).
78. C. S. Chen, D. E. Ingber, Tensegrity and mechanoregulation: from skeleton to cytoskeleton. *Osteoarthritis and cartilage* **7**, 81 (Jan, 1999).
79. C. Galli, S. Guizzardi, G. Passeri, G. M. Macaluso, R. Scandroglio, Life on the wire: on tensegrity and force balance in cells. *Acta bio-medica : Atenei Parmensis* **76**, 5 (Apr, 2005).
80. D. E. Ingber, Integrins, tensegrity, and mechanotransduction. *Gravitational and space biology bulletin : publication of the American Society for Gravitational and Space Biology* **10**, 49 (Jun, 1997).
81. K. Y. Volokh, On tensegrity in cell mechanics. *Molecular & cellular biomechanics : MCB* **8**, 195 (Sep, 2011).
82. M. Wall *et al.*, Key developments that impacted the field of mechanobiology and mechanotransduction. *Journal of orthopaedic research : official publication of the Orthopaedic Research Society* **36**, 605 (Feb, 2018).
83. F. T. Bosman, I. Stamenkovic, Functional structure and composition of the extracellular matrix. *Journal of Pathology* **200**, 423 (Jul, 2003).

84. Y. C. Fong *et al.*, Transforming growth factor-beta1 increases cell migration and beta1 integrin up-regulation in human lung cancer cells. *Lung Cancer* **64**, 13 (Apr, 2009).
85. C. Margadant, A. Sonnenberg, Integrin-TGF-beta crosstalk in fibrosis, cancer and wound healing. *EMBO reports* **11**, 97 (Feb, 2010).
86. M. A. Cichon, D. C. Radisky, Extracellular matrix as a contextual determinant of transforming growth factor-beta signaling in epithelial-mesenchymal transition and in cancer. *Cell adhesion & migration* **8**, 588 (2014).
87. D. Bianconi, M. Unseld, G. W. Prager, Integrins in the Spotlight of Cancer. *International journal of molecular sciences* **17**, (Dec 6, 2016).
88. Z. Khan, J. F. Marshall, The role of integrins in TGFbeta activation in the tumour stroma. *Cell and tissue research* **365**, 657 (Sep, 2016).
89. A. Moustakas, C. H. Heldin, Dynamic control of TGF-beta signaling and its links to the cytoskeleton. *FEBS letters* **582**, 2051 (Jun 18, 2008).
90. A. Moustakas, C. Stournaras, Regulation of actin organisation by TGF-beta in H-ras-transformed fibroblasts. *Journal of cell science* **112 (Pt 8)**, 1169 (Apr, 1999).
91. S. Edlund, M. Landstrom, C. H. Heldin, P. Aspenstrom, Transforming growth factor-beta-induced mobilization of actin cytoskeleton requires signaling by small GTPases Cdc42 and RhoA. *Molecular biology of the cell* **13**, 902 (Mar, 2002).
92. N. A. Bhowmick *et al.*, Transforming growth factor-beta1 mediates epithelial to mesenchymal transdifferentiation through a RhoA-dependent mechanism. *Molecular biology of the cell* **12**, 27 (Jan, 2001).
93. A. Rothballer, U. Kutay, The diverse functional LINCs of the nuclear envelope to the cytoskeleton and chromatin. *Chromosoma* **122**, 415 (Oct, 2013).
94. E. Konde *et al.*, Structural analysis of the Smad2-MAN1 interaction that regulates transforming growth factor-beta signaling at the inner nuclear membrane. *Biochemistry* **49**, 8020 (Sep 21, 2010).
95. J. H. Van Berlo *et al.*, A-type lamins are essential for TGF-beta1 induced PP2A to dephosphorylate transcription factors. *Human molecular genetics* **14**, 2839 (Oct 1, 2005).
96. C. Le Dour *et al.*, Extracellular matrix remodeling and transforming growth factor-beta signaling abnormalities induced by lamin A/C variants that cause lipodystrophy. *Journal of lipid research* **58**, 151 (Jan, 2017).
97. T. P. O'Brien *et al.*, Genome function and nuclear architecture: from gene expression to nanoscience. *Genome research* **13**, 1029 (Jun, 2003).
98. T. Pederson, The nuclear physique. *International review of cell and molecular biology* **307**, 1 (2014).
99. T. Pederson, M. C. King, J. F. Marko, Forces, fluctuations, and self-organization in the nucleus. *Molecular biology of the cell* **26**, 3915 (Nov 5, 2015).
100. A. Aranda-Anzaldo, M. A. Dent, A. Martinez-Gomez, The higher-order structure in the cells nucleus as the structural basis of the post-mitotic state. *Progress in biophysics and molecular biology* **114**, 137 (May, 2014).
101. C. Uhler, G. V. Shivashankar, Regulation of genome organization and gene expression by nuclear mechanotransduction. *Nature reviews. Molecular cell biology* **18**, 717 (Dec, 2017).
102. M. H. Barcellos-Hoff, F. A. Cucinotta, New tricks for an old fox: impact of TGFbeta on the DNA damage response and genomic stability. *Science signaling* **7**, re5 (Sep 2, 2014).
103. J. Lee, M. R. Kim, H. J. Kim, Y. S. An, J. Y. Yi, TGF-beta1 accelerates the DNA damage response in epithelial cells via Smad signaling. *Biochemical and biophysical research communications* **476**, 420 (Aug 5, 2016).
104. D. Pal *et al.*, TGF-beta reduces DNA ds-break repair mechanisms to heighten genetic diversity and adaptability of CD44+/CD24- cancer cells. *Elife* **6**, (Jan 16, 2017).

105. R. Elston, G. J. Inman, Crosstalk between p53 and TGF-beta Signalling. *Journal of signal transduction* **2012**, 294097 (2012).
106. L. Centurione, F. B. Aiello, DnA Repair and Cytokines: TGF-beta, IL-6, and Thrombopoietin as Different Biomarkers of Radioresistance. *Frontiers in oncology* **6**, (Jul 22, 2016).
107. F. J. Lopez-Diaz *et al.*, Coordinate Transcriptional and Translational Repression of p53 by TGF-beta 1 Impairs the Stress Response. *Molecular cell* **50**, 552 (May 23, 2013).
108. M. Makale, Cellular mechanobiology and cancer metastasis. *Birth defects research. Part C, Embryo today : reviews* **81**, 329 (Dec, 2007).
109. M. W. Pickup, J. K. Mouw, V. M. Weaver, The extracellular matrix modulates the hallmarks of cancer. *EMBO reports* **15**, 1243 (Dec, 2014).
110. M. J. Binder, S. McCoombe, E. D. Williams, D. R. McCulloch, A. C. Ward, The extracellular matrix in cancer progression: Role of hyalectan proteoglycans and ADAMTS enzymes. *Cancer letters* **385**, 55 (Jan 28, 2017).
111. R. Malik, P. I. Lelkes, E. Cukierman, Biomechanical and biochemical remodeling of stromal extracellular matrix in cancer. *Trends Biotechnol* **33**, 230 (Apr, 2015).
112. C. Neuzillet *et al.*, Targeting the TGFbeta pathway for cancer therapy. *Pharmacology & therapeutics* **147**, 22 (Mar, 2015).