

Aberrant mechanical microenvironment in lung cancer probed at the micro- and nano-scales

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Abstract

The lung is a moderately soft organ with unique mechanical properties that are necessary for breathing. However, the appearance of a desmoplastic stroma rich in activated fibroblasts/myofibroblasts and pro-fibrotic extracellular matrix (ECM) components in lung tumors strongly suggests that the cellular microenvironment becomes abnormally stiff in lung cancer. To test this hypothesis, we have analyzed the expression of fibrillar collagens (a major pro-fibrotic ECM component) *in vivo* by picrosirius red staining, and have assessed the mechanical consequences of enhanced collagen density *ex-vivo* by atomic force microscopy (AFM). Likewise, we have determined the fraction of activated fibroblasts *in vivo*, and the mechanical consequences of such activation in culture at the nano- and micro-scale by AFM. Finally, we have examined cell-ECM mechanical interactions in fibroblasts at the nano-scale with microfabricated flat-ended-AFM tips designed to mimic few cell-ECM adhesion sites. The biological consequences of aberrant cell-ECM mechanical interactions in tumors were further analyzed in terms of fibroblast accumulation, which is a major hallmark of solid tumors in the lung and other organs.

Normal lung parenchyma exhibited weak collagen staining and short collagen fibers, whereas tumor samples exhibited abundant collagen staining that was frequently organized into long and straight fibril bundles indicative of mechanical tension. Likewise, fibril bundles were often organized in a parallel fashion (see dashed squares) (1), which has been previously shown to render stiffer tissues. To examine the mechanical effects of increased collagen deposition, we probed the Young's elastic modulus (E) of collagen gels with increasing concentration by AFM. E scaled with collagen density according c to $E \sim c^2$. This dependence is well captured by current models of semiflexible semidiluted polymers in which the fibril length is larger than the pore or mesh size (2).

In normal lung parenchyma, fibroblasts were sparsely located, and α -SMA+ cells corresponding to smooth muscle cells were largely restricted to the perivascularity (see black arrows). In contrast, lung tumors exhibited a stroma rich in peritumoral α -SMA+ fibroblasts. These features were used to assess the relative amount of tumor stroma as the percentage of α -SMA+ area, which elicited ~25% (1). To mimic fibroblast activation in culture, most fibroblasts required exogenous TGF- β 1, which is a potent fibrotic cytokine commonly upregulated in the tumor microenvironment. Treatment of fibroblasts with TGF- β 1 induced *de novo* expression of α -SMA incorporated into stress fibers in culture. Concomitantly to these cytoskeletal alterations, TGF- β 1 treatment increased the Young's modulus more than 2-fold with respect to untreated cells.

To investigate the critical molecular machinery involved in cell-ECM force transmission, we microfabricated flat-ended AFM tips with a constant cross-section area of $\sim 1 \mu\text{m}^2$, and coated them with an RGD peptide, which is an adhesive domain found in many ECM components that is specifically recognized by ECM integrin receptors. The RGD-coated flat-ended tip was brought to contact with the surface of a single fibroblasts up to a moderate force, hold for 30 s to enable the formation of focal adhesion precursors, and retracted until the contact was lost. We found a marked increase in both cell stiffness and cell-ECM adhesion when using RGD-coated tips, but not RGE or other non-integrin specific coatings. Such cell stiffening and adhesion strengthening were abrogated upon inhibition of actin but not microtubule polymerization, revealing that local fibroblast mechanoresponses requires integrin-mediated rearrangements of the actin cytoskeleton (3).

To examine the pathological consequences of abnormal integrin mechanosensing in fibroblasts, we analyzed how extracellular stiffening comparable to that expected within the tumor microenvironment altered fibroblast behavior in an integrin-specific fashion. We found that fibroblast density markedly increased in gels with a tumor-like rigidity compared to soft gels with normal-like rigidity values. Remarkably, such cell density increase was abrogated upon inhibition of β 1 integrin mechanosensing through FAKpY397, which is the most abundant component of fibroblast integrin receptors (1).

In summary, we have collected evidence supporting that the tumor microenvironment is much stiffer than the normal lung parenchyma and that activated fibroblasts are largely responsible for such

tissue hardening. Of note, we have also obtained evidence of a positive feedback loop in which activated fibroblasts increase tissue hardening, which in turns stimulates fibroblast accumulation in a beta1-integrin dependent fashion, which is a hallmark of lung cancer.

References

1. Puig M, Lugo R, Gabasa M, Giménez A, Velásquez A, Galgoczy R, et al. Matrix stiffening and β 1 integrin drive fibroblast accumulation in lung cancer in a subtype-dependent fashion. *Mol Cancer Res*. 2014;(in press).
2. Alcaraz J, Mori H, Ghajar CM, Brownfield D, Galgoczy R, Bissell MJ. Collective epithelial cell invasion overcomes mechanical barriers of collagenous extracellular matrix by a narrow tube-like geometry and MMP14-dependent local softening. *Integr Biol (Camb)*. 2011;3:1153-66.
3. Acerbi I, Luque T, Gimenez A, Puig M, Reguart N, Farre R, et al. Integrin-specific mechanoresponses to compression and extension probed by cylindrical flat-ended AFM tips in lung cells. *PLoS One*. 2012;7:e32261.