Collagens are substantial constituents of the extracellular matrix (ECM) of every organ system, and virtually every cell in a tissue has the opportunity to interact with fibrillar or basement membrane collagens in the context of tissue development, remodeling, or homeostasis. Tissue architecture is governed by the structure imparted by the ECM; maintenance of this architecture is a barrier to malignant transformation. However, documentation that dysfunctional or inappropriate ECM contributes directly to tumor development is uncommon.

Collagen secretion, fibrillogenesis, and deposition are the result of a precise series of biochemical events that are facilitated by extracellular adaptor proteins, including proteoglycans (e.g., fibromodulin, lumican, and decorin) and matricellular proteins such as secreted protein acidic and rich in cysteine (SPARC; ref. 1). Of these extracellular adaptor proteins, SPARC has received considerable attention in the context of tumor development and progression. SPARC expression is coincident with collagen secretion, and global loss of SPARC expression in mice results in defective collagen deposition and a less-robust ECM in normal tissues and malignant lesions. The molecular consequences of loss of SPARC expression in tumors is still under investigation but is highly relevant, as the SPARC gene is frequently subject to epigenetic regulation in epithelial tumor cells (2). Although many activities have been ascribed to SPARC (3), a definition of how SPARC functions in the tumor microenvironment remains to be determined.

Although a unifying principle of SPARC function in the tumor microenvironment remains elusive, the Colombo laboratory has in a series of recent studies (4) demonstrated that SPARC is a critical component of the complex mechanisms that control immune cell recruitment, activation, and proliferation. Furthermore, SPARC is required for appropriate assembly of germinal centers where expansion of adaptive immune cells occurs. Given these observations, they set out to investigate whether altered ECM deposition exacerbated lymphoma development in the context of autoimmunity, which predisposes for lymphoid malignancies. In particular, autoantigen-driven clonal expansion is a frequent occurrence in chronic lymphocytic leukemia (SLL), although contributing factors required for malignant lymphoproliferation are unclear. Sangaletti and colleagues (5) utilized their knowledge of the changes in secondary lymphoid organ (SLO) architecture in Sparc−/− animals to assess the contribution of collagen signaling to autoimmunity-induced lymphoma. Fas-mutant lpr/lpr mice are prone to autoimmunity as a result of defective apoptosis in autoreactive B-cell clones (6). The authors crossed lpr/lpr and Sparc−/− double-mutant mice showed accelerated autoimmune-driven lymphoproliferation. Lymphoid tissue from double-mutant but not lpr/lpr/Sparc−/− mice generated subcutaneous tumors in recipient immunocompromised mice and displayed diffuse proliferation of lymphoid elements similar to the originating lesions, confirming their malignant nature.

The development of a functional adaptive immune response requires the coordinated and selective interaction of immune cells in SLOs. The opportunity for these interactions is governed in part by the architecture of the tissue. The authors observed that collagen deposition in the spleen was dysregulated and there were disorganized stromal boundaries between white pulp follicles and red pulp follicles in double-mutant but not lpr/lpr mice. These structural changes appear to be a critical factor in the proliferative expansion of CD5+B cells, as there was no basal difference in B-cell proliferation in Sparc−/− and Sparc+/+ animals. To better understand how...
the alteration in the microenvironment affects lymphoid expansion, the authors evaluated lymphoid tissue for myeloid cells, which can release B-cell trophic factors. They identified that Ly-6G+ neutrophils were more numerous in double-mutant spleens and that these cells were in close contact with expanding clonal B cells. Furthermore, they found that neutrophils from double-mutant mice underwent NETosis at an increased frequency than those found in lpr/lpr mice. NETosis is a process by which neutrophils die and extrude a neutrophil extracellular trap (NET) consisting of chromatin and cytoplasmic content that stimulate an inflammatory response (7). This process can enhance immune recognition of pathogens or in the setting of autoimmunity stimulate autoantigen release. Double-mutant mice showed an increase in B cells in contact with NETs in splenic tissue. Additional in vitro culture experiments demonstrated that NETotic neutrophil cell death but not apoptotic or necrotic cell death stimulated B-cell expansion via NF-κB activation.

To further define how myeloid cells contribute to the observed elevation in lymphoproliferation, the authors examined the gene expression profile of neutrophils from double-mutant and lpr/lpr;Sparc+/- mice. Neutrophils from Sparc+/- animals showed an increase in several genes related to the IFN signature, suggesting that neutrophils from Sparc-deficient animals received more robust priming for NETosis. The critical link between microenvironmental NET-mediated expansion of autoreactive B cells and defects in collagen deposition came when the authors examined the expression of LAIR-1, a collagen receptor on neutrophils that has an inhibitory function. LAIR-1 expression was decreased in neutrophils isolated from double-mutant animals. To provide a functional link between ECM composition and NETosis, the authors showed that neutrophils isolated from double-mutant mice robustly underwent NETosis when plated on poly-D-lysine but not collagen. Further evidence of the importance of LAIR-1 activity as an inhibitory signal for NETosis was provided by RNAi-mediated suppression of LAIR1 in granulocytes isolated from wild-type mice. In the absence of LAIR-1, these cells underwent NETosis regardless of substrate, whereas NETosis was inhibited by collagen in control cells.

These results strongly implicate the deficiency in collagen deposition as a major contributor to autoreactive lymphoma genesis in lpr/lpr;Sparc+/- animals and are a striking example of the importance of ECM architecture in maintaining tissue homeostasis. To determine the clinical relevance of these observations, the authors searched for human correlates. They examined the expression of SPARC in a series of non-Hodgkin B-cell lymphomas and found that CLL showed consistent and significant downregulation of SPARC at the mRNA and protein level. Importantly, the absence of SPARC expression correlated with reduced collagen and LAIR-1 expression. Furthermore, tissue from patients with CLL showed higher frequency of stromal disorganization and NETosis, histologic features reminiscent of the microenvironment of spleens in lpr/lpr;Sparc+/- mice. These observations suggest that alterations in stromal features, including SPARC expression, may be an early event that promotes malignant B-cell lymphomagenesis.

These studies add further intrigue to the growing interest in how ECM and matricellular proteins contribute to the tumor microenvironment. Questions remain regarding control of SPARC expression in SLOs in patients with CLL and whether other B-cell malignancies show alterations in collagen signaling. For example, in contrast to the study by Sangaletti and colleagues (5), a recent report (8) suggests that productive collagen signaling is a driving factor in malignant Hodgkin/Reed-Sternberg cells in Hodgkin lymphoma. This study reports that an Epstein-Barr virus-encoded protein (latent membrane protein-1) upregulates the expression of the collagen receptor tyrosine kinase discoidin domain receptor 1 (DDR1), which drove collagen-mediated lymphoma proliferation and provided protection from apoptosis. Intriguingly, SPARC may be a contributing factor in this setting as well. SPARC and the DDRs share the same binding epitope on collagen (9, 10), and thus one of the functions of SPARC might be to limit collagen-mediated activation of DDRs. Therefore, loss of SPARC in the tumor microenvironment can potentially contribute in multiple defined ways to tumor development and progression. Overall, it is clear that the collagen network is an active participant in the signaling that prevents or promotes tumor development and progression. As our understanding of these collagen-mediated signaling events expands, new opportunities for therapeutic intervention will be discovered and the tools to test these strategies will be in hand.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Faulty ECM Signaling Facilitates Autoimmune Lymphomagenesis

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