

contrast the ECM within a normal simple epithelial tissue with that found within a pathologically modified tissue, as exemplified in aged tissue, wounded or fibrotic tissue and tumors. We particularly focus on the composition and architecture of the ECM and interactions with its cellular constituents, and describe in detail common post-translational modifications that evoke defined topological and viscoelasticity changes in the tissue. We thereafter discuss the functional consequences of ECM remodeling on cellular behaviors including altered GF sensitivity elicited by changes in ECM tension. Owing to space limitations and because the basement membrane (BM) is a unique ECM that has been reviewed in detail elsewhere (LeBleu et al., 2007), we focus here on the interstitial stroma of simple glandular epithelial tissues. We complete our review with a brief discussion of the application of natural and synthetic ECMs that can be used to either recapitulate the interstitial ECM in culture to study tissue behaviors or to deconstruct and analyze how specific ECM parameters (stiffness, fiber orientation, ligand presentation, dimensionality) provoke specific cellular behaviors.

Bits and pieces – molecular composition of the ECM

The ECM is composed of two main classes of macromolecules: proteoglycans (PGs) and fibrous proteins (see Boxes 1 and 2) (Jarvelainen et al., 2009; Schaefer and Schaefer, 2010). The main fibrous ECM proteins are collagens, elastins, fibronectins and laminins (see panel 1 of the poster) (Alberts et al., 2007). PGs fill the majority of the extracellular interstitial space within the tissue in the form of a hydrated gel (Box 1) (for details, see Jarvelainen et al., 2009). PGs have a wide variety of functions that reflect their unique buffering, hydration, binding and force-resistance properties. For example, in the kidney glomerular BM, perlecan has a role in glomerular filtration (Harvey and Miner, 2008; Morita et al., 2005). By contrast, in ductal epithelial tissues, decorin, biglycan and lumican associate with collagen fibers to generate a molecular structure within the ECM that is essential for mechanical buffering and hydration and that, by binding GFs, provides an easy, enzymatically accessible repository for these factors (Iozzo and Murdoch, 1996).

Collagen is the most abundant fibrous protein within the interstitial ECM and constitutes up to 30% of the total protein mass of a multicellular animal. Collagens, which constitute the main structural element of the ECM, provide tensile strength, regulate cell adhesion, support chemotaxis and migration, and direct tissue development (Rozario and DeSimone, 2010).

Box 1. Structure and function of proteoglycans

Proteoglycans (PGs) are composed of glycosaminoglycan (GAG) chains covalently linked to a specific protein core (with the exception of hyaluronic acid) (Iozzo and Murdoch, 1996; Schaefer and Schaefer, 2010). PGs have been classified according to their core proteins, localization and GAG composition. The three main families are: small leucine-rich proteoglycans (SLRPs), modular proteoglycans and cell-surface proteoglycans (Schaefer and Schaefer, 2010). The GAG chains on the protein core are unbranched polysaccharide chains composed of repeating disaccharide units [sulfated *N*-acetylglucosamine or *N*-acetylgalactosamine, *D*-glucuronic or *L*-iduronic acid and galactose (–4 *N*-acetylglucosamine- β 1,3-galactose- β 1)] that can be divided further into sulfated (chondroitin sulfate, heparan sulfate and keratan sulfate) and non-sulfated (hyaluronic acid) GAGs (Schaefer and Schaefer, 2010). These molecules are extremely hydrophilic and, accordingly, adopt highly extended conformations that are essential for hydrogel formation and that enable matrices that are formed by these molecules to withstand high compressive forces. Many genetic diseases have been linked to mutations in PG genes (Jarvelainen et al., 2009; Schaefer and Iozzo, 2008). SLRPs have been involved in multiple signaling pathways including binding to and activation of epidermal growth factor receptor (EGFR), insulin-like growth factor 1 receptor (IGFIR) and low-density lipoprotein-receptor-related protein 1 (LRP1), regulation of inflammatory response reaction, binding to and activation of TGF β (Goldoni and Iozzo, 2008; Schaefer and Iozzo, 2008; Schaefer and Schaefer, 2010). Modular PGs can modulate cell adhesion, migration and proliferation (Schaefer and Schaefer, 2010). Basement membrane modular PGs (perlecan, agrin and collagen type XVIII) have a dual function as pro- and anti-angiogenic factors (Iozzo et al., 2009). Cell-surface PGs (syndecans and glypicans) can act as co-receptor facilitating ligand encounters with signaling receptors (Schaefer and Schaefer, 2010).

The bulk of interstitial collagen is transcribed and secreted by fibroblasts that either reside in the stroma or are recruited to it from neighboring tissues (De Wever et al., 2008). By exerting tension on the matrix, fibroblasts are able to organize collagen fibrils into sheets and cables and, thus, can dramatically influence the alignment of collagen fibers. Although within a given tissue, collagen fibers are generally a heterogeneous mix of different types, one type of collagen usually predominates.

Collagen associates with elastin, another major ECM fiber. Elastin fibers provide recoil to tissues that undergo repeated stretch. Importantly, elastin stretch is crucially limited by tight association with collagen fibrils (Wise and Weiss, 2009). Secreted tropoelastin (the precursor of elastin) molecules assemble into fibers and become highly crosslinked to one another via their lysine residues by members of the lysyl oxidase (LOX) enzyme family, which include LOX and LOXL (Lucero and Kagan, 2006). Elastin fibers are covered by glycoprotein microfibrils, mainly fibrillins, which are also essential for the integrity of the elastin fiber (Wise and Weiss, 2009).

A third fibrous protein, fibronectin (FN) is intimately involved in directing the organization of the interstitial ECM and, additionally, has a crucial role in mediating cell attachment and function. FN can be stretched several times over its resting length by cellular traction forces (Smith et al., 2007). Such force-dependent unfolding of FN exposes cryptic integrin-binding sites within the molecule that result in

pleiotropic changes in cellular behavior and implicate FN as an extracellular mechano-regulator (Smith et al., 2007). Indeed, 'tensed' FN modulates the catch bond 'force-activation' and adhesion assembly of α 5 β 1-integrin through exposure of its synergy site (Friedland et al., 2009). FN is also important for cell migration during development and has been implicated in cardiovascular disease and tumor metastasis (Rozario and DeSimone, 2010; Tsang et al., 2010). Like FN, other ECM proteins such as tenascin exert pleiotropic effects on cellular behavior, including the promotion of fibroblast migration during wound healing (Trebaul et al., 2007; Tucker and Chiquet-Ehrismann, 2009). Indeed, levels of tenascins C and W are elevated in the stroma of some transformed tissues where they can inhibit the interaction between syndecan4 and FN to promote tumor growth and metastasis (Tucker and Chiquet-Ehrismann, 2009).

The definition of normal – the ECM and tissue homeostasis

Normal glandular epithelial tissues are composed of a simple layer of epithelial cells that adopt apical–basal polarity, where the basal side contacts the BM and the apical side is opposite the fluid-filled lumen. In some glandular epithelium there is a basal or myoepithelial cell layer that separates the luminal epithelium from the interstitial ECM (Barsky and Karlin, 2005). Epithelial tissue homeostasis depends on the maintenance of tissue organization and a dynamic dialogue with

a surrounding stroma composed primarily of non-activated fibroblasts and adipocytes, and a steady-state population of transiting, non-stimulated leukocytes (Ronnov-Jessen et al., 1996). Thus, non-activated tissue fibroblasts secrete and organize type I and III collagens, elastin, fibronectin, tenascin and a repertoire of PGs (hyaluronic acid and decorin), which all maintain the structural and functional integrity of the interstitial ECM. Most glandular epithelial tissues including breast, saliva gland, lung, and prostate are in a state of tensional homeostasis so that their normal state is highly mechanically compliant (Paszek and Weaver, 2004). The ECM in a compliant tissue is composed of a relaxed meshwork of type I and III collagens and elastin that, together with FN, form a relaxed network of fibers that are surrounded by and embedded in a hydrogel of glycosaminoglycan-chain-containing PGs (Bosman and Stamenkovic, 2003). Consequently, the relaxed network of collagen and elastin fibers allow the healthy ECM to resist a wide range of tensile stresses. A functionally competent normal tissue can also easily resist compressive stresses because of the binding of the hydrated glycosaminoglycan (GAG) network to the fibrous ECM molecules (Scott, 2003). Thus, the tissue ECM is a highly dynamic entity that continuously undergoes regulated remodeling, whose precise orchestration is crucial to the maintenance of normal function (Egeblad et al., 2010; Kass et al., 2007). Tissue homeostasis is mediated by the coordinated secretion of fibroblast metalloproteinases (MMPs) (Mott and Werb, 2004); this is counterbalanced by tissue inhibitors of metalloproteinases (TIMPs) (Cruz-Munoz and Khokha, 2008) and the controlled activity of other enzymes, such as LOX, and also transglutaminases that crosslink and, consequently, stiffen the ECM (Lucero and Kagan, 2006). A plethora of GFs that are bound to the ECM direct these processes (Friedl, 2010; Hynes, 2009; Macri et al., 2007; Murakami et al., 2008; Oehrl and Panayotou, 2008). These ECM-bound GFs differentially modulate cell growth and migration and, when released, comprise part of a tightly controlled feedback circuit that is essential for normal tissue homeostasis (Hynes, 2009).

Stiffening up – the ECM and tissue aging

As a tissue ages the levels of junctional proteins such as cadherin, catenin or occludin decrease and this loss can compromise junctional integrity as revealed by the appearance of gaps between the epithelial cells (Akintola et al., 2008; Bolognia, 1995). Old tissue is also characterized by a thinning of the BM, probably

Box 2. Collagen and fibronectin synthesis

To date, 28 types of collagen have been identified in vertebrates (Gordon and Hahn, 2010). The majority of collagen molecules form a triple-stranded helix that subsequently can assemble into supramolecular complexes, such as fibrils and networks, depending on the type of collagen. Fibrous collagens form the backbone of the collagen fibril bundles within the interstitial tissue stroma, whereas network collagens are incorporated into the basal membrane (BM). Synthesis of collagen type I involves a number of enzymatic post-translational modifications (Gordon and Hahn, 2010; Myllyharju and Kivirikko, 2004), mainly the hydroxylation of proline and lysine residues, glycosylation of lysine and the cleavage of N- and C-terminal propeptides. Following their cleavage, collagen fibrils are strengthened by the covalent crosslinking between lysine residues of the constituent collagen molecules by lysyl oxidases (LOX) (Myllyharju and Kivirikko, 2004; Robins, 2007).

FN is secreted as a dimer joined by two C-terminal disulfide bonds and has several binding sites to other FN dimers, to collagen, to heparin and also to cell-surface integrin receptors (Pankov and Yamada, 2002). Cell-surface binding of the soluble FN dimer is essential for its assembly into longer fibrils. Moreover, cell contraction through the actomyosin cytoskeleton and the resulting integrin clustering promotes FN–fibril assembly by exposing cryptic binding sites, thus allowing them to bind one another (Leiss et al., 2008; Mao and Schwarzbauer, 2005; Vakonakis and Campbell, 2007).

because of elevated MMP-mediated degradation and reduced BM protein synthesis (Callaghan and Wilhelm, 2008). Moreover, the resident fibroblasts in aged tissues are growth-arrested and resistant to apoptotic cues, which is indicative of senescence (Campisi and d'Adda di Fagagna, 2007). Indeed, senescent fibroblasts typically express elevated levels of FN, MMPs, GFs, interleukins and cytokines, as well as high levels of the plasminogen activator inhibitor (PAI) (Coppe et al., 2010) and mitochondrial-related reactive oxygen species (ROS) (Untergasser et al., 2005) and, as a result, are frequently in a state of chronic inflammation. Indeed, the combination of chronic inflammation and elevated MMPs, PAI and ROS destroy the integrity of the elastin network and modify the collagen fiber network, whereas reduced levels of tissue-associated GAGs compromise the integrity of the BM (Callaghan and Wilhelm, 2008; Calleja-Agius et al., 2007; Nomura, 2006). Nevertheless, and somewhat paradoxically, in an aging tissue, collagen fibers are frequently – inappropriately – crosslinked through glycation, by byproducts of lipid oxidation and through exposure to UV light (Robins, 2007). The combination of elevated and inappropriate collagen crosslinking contributes to tissue stiffening so that an aged tissue is mechanically weaker and less elastic but also more rigid than a young tissue (Calleja-Agius et al., 2007; Robins, 2007). This aberrant mechanical state can severely compromise ECM organization, and modify epithelial organization and function, potentially promoting age-related diseases such as cancer (Coppe et al., 2010; Freund et al., 2010; Sprenger et al., 2008).

Tensional homeostasis and fibrosis

Acute injury activates the fibrogenic machinery and induces wound healing. One of the first

events that characterize a wound response is vascular damage and the formation of a fibrin clot, which stimulates monocyte infiltration to the damaged ECM. Upon binding to ECM-degradation products and cytokines, monocytes rapidly differentiate into macrophages (Clark, 2001). These activated macrophages, in turn, secrete and release multiple GFs, MMPs and cytokines that promote angiogenesis and stimulate fibroblast migration and proliferation (Schultz and Wysocki, 2009). Thereafter, recruited fibroblasts begin to synthesize and deposit large quantities of ECM proteins, including collagen type I and III, FN and hyaluronic acid. The elevated mechanical stress associated with this profound ECM deposition can induce the transdifferentiation of fibroblasts and other tissue-resident cells – i.e. epithelial-to-mesenchymal transition (EMT) of epithelial cells – or of circulating bone marrow-derived mesenchymal stem cells into myofibroblasts (Schultz and Wysocki, 2009; Velnar et al., 2009). Myofibroblasts, which have a high capacity to synthesize ECM components and are highly contractile, can promote the formation of large, rigid collagen bundles that, if crosslinked by LOX enzymes, mechanically strengthen and stiffen the tissue (Szauder et al., 2005). This wounded ‘stiffened’ microenvironment disrupts the BM that surrounds the epithelium and compromises tissue integrity with loss of apical–basal polarity and destabilized cell–cell adhesions. The remodeled ECM also promotes the directional migration of cells within the tissue towards the wound site (Schafer and Werner, 2008). In some instances, the release of transforming growth factor β (TGF- β) by tension and MMPs induces EMT of the resident epithelium (Schultz and Wysocki, 2009; Wipff et al., 2007; Xu et al., 2009). In a healthy tissue, once the wound has been repopulated, strict

feedback mechanisms are initiated that ensure restoration of tissue homeostasis and resolution of fibrosis (Schultz and Wysocki, 2009; Velnar et al., 2009). Under extreme conditions, such as repeated injury or when normal feedback mechanisms are compromised, continuous ECM synthesis, deposition and remodeling ensue and myofibroblasts remain, in which TIMP production prevails over MMP synthesis. These aberrant conditions promote chronic vascular remodeling and enhanced ECM crosslinking that eventually leads to aberrant fibrosis and an inability of the tissue to heal properly. This aberrant wound healing scenario is characterized by the altered mechanical stability and reduced elasticity that is typical of scarred tissue (Kisseleva and Brenner, 2008). In extreme cases, a chronic wound can also promote a tumor phenotype (De Wever et al., 2008).

Tumors – a tough situation

Cancer is the loss of tissue organization and aberrant behavior of the cellular components. Cell transformation results from genetic mutations and epigenetic alterations. Yet, tumors have also been likened to wounds that fail to heal (Schafer and Werner, 2008). Thus, the tumor stroma exhibits some of the characteristics found in an unresolved wound (Bissell and Radisky, 2001). For example, tumors are characteristically stiffer than the surrounding normal tissue. The stiffening of tumors is induced by ECM deposition and remodeling by resident fibroblasts, and by increased contractility of the transformed epithelium (Butcher et al., 2009; Levental et al., 2009). Moreover, chemokines and GFs (De Wever et al., 2008) induce inflammation and modify the repertoire of infiltrating T lymphocytes (Tan and Coussens, 2007). Tissue inflammation potentiates stromal fibroblast activation and induces their trans-differentiation into myofibroblasts, thus exacerbating and promoting tissue desmoplasia (De Wever et al., 2008; Desmouliere et al., 2004). Myofibroblasts deposit copious quantities of ECM proteins, secrete GFs and exert strong contraction forces on the ECM (De Wever et al., 2008; Desmouliere et al., 2004). As a consequence, newly deposited and remodeled collagen and elastin fibers are reoriented and, thereafter, crosslinked by LOX and transglutaminase, thus generating larger, more-rigid fibrils that further stiffen the tissue ECM (Butcher et al., 2009; Erler and Weaver, 2009; Levental et al., 2009; Lucero and Kagan, 2006; Payne et al., 2007; Rodriguez et al., 2008). MMPs, which are secreted and activated by tumor cells and by myofibroblasts (De Wever et al., 2008; Kessenbrock et al., 2010), also remodel the BM surrounding the tumor and release and activate

ECM-embedded GFs (Bosman and Stamenkovic, 2003; Kessenbrock et al., 2010). The release of GFs, including vascular endothelial growth factor (VEGF), enhances vascular permeability and promotes new vessel growth, which generates interstitial tissue pressure. Thus, an amplifying circuitry between tumor-associated ECM stiffening, an ensuing reciprocal ECM resistance that is induced by resident tumor cells, and myoepithelial and cell-generated contractility act as a vicious, positive-feedback loop to potentiate tumor growth and survival. This induces angiogenesis and invasion and, eventually, fosters metastasis (Butcher et al., 2009; Erler and Weaver, 2009; Paszek and Weaver, 2004; Paszek et al., 2005).

Where do we go from here?

Challenges encountered with natural and synthetic ECMs

Considering the importance of the ECM to so many fundamental cellular processes, a myriad of tissue-culture models have been developed to study the interplay between its biochemical and biophysical properties, and to understand the molecular origins of cellular behaviors regulated by ECM ligation. With respect to assessing the fundamental nature of cell adhesion and its effects on cell behavior, the majority of cancer researchers have relied on coating tissue culture dishes (whether plastic or glass) with purified preparations or mixtures of ECM proteins in order to obtain 2D monolayers (Kuschel et al., 2006). To address the issue of ECM rigidity, functionalized polyacrylamide (PA) gels crosslinked with reconstituted basement membrane (rBM) – generated from Engelbreth-Holm-Swarm mouse carcinoma (commercially available as Matrigel™), collagen type I, FN or ECM peptides – has become the standard approach (Johnson et al., 2007; Pelham and Wang, 1997). Yet, none of these strategies faithfully recapitulates the behavior of cells within tissues, which demand not only a 3D format, but an ECM that can be readily remodeled. To address the aspect of 3D and ECM remodeling, researchers have used natural ECM and reconstituted ECM gels to recapitulate specific aspects of tissue-specific differentiation and architecture (see poster, panel 3). For instance, the rBM, which mimics some of the biochemical and biophysical properties of endogenous epithelial basement membranes, has been used frequently in 3D organotypic culture assays, for xenograft manipulations or tissue engineering, and to study tissue-specific morphogenesis (e.g. branching, acini formation) and differentiation (Kleinman and Martin, 2005; Kleinman et al., 1986). Unfortunately, BM preparations such as Matrigel™, although useful for studying normal

epithelial or endothelial behavior and to distinguish between the ‘normal’ and ‘malignant’ behavior of some tissues, has a complex and rudimentarily defined composition, and fails to reconstruct the physical state of the native interstitial ECM. Fibrin has also been used as natural biodegradable scaffold with reasonable success in vascular tissue engineering, but lacks the mechanical strength and durability of native interstitial ECM (Blomback and Bark, 2004; Shaikh et al., 2008). By contrast, type I collagen is reasonably useful and can be combined with rBM, purified laminin or FN to reconstitute some of the biological aspects of normal and diseased interstitial ECM (Friess, 1998; Gudjonsson et al., 2002). Moreover, collagen type I readily assembles into a mechanically tense network of fibrils that can be oriented, functionally modified, and enzymatically or chemically crosslinked and stiffened. Thus collagen I gels are useful substrates to assess the role of collagen and FN stiffness, and organization on the pathogenesis of tumor progression and invasion (Levental et al., 2009; Provenzano et al., 2009). Nevertheless, collagen gels are quite heterogeneous, and modifying their architecture changes their organization, pore size and ligand concentration, thereby complicating the interpretation of data generated from experiments conducted by using this natural scaffold (Johnson et al., 2007). To overcome this issue, tissue engineers and biomaterial specialists have generated denuded ECM scaffold from various tissues (Macchiaroni et al., 2008). These scaffolds, combined with colonies of seeded stem cells, can reconstitute normal tissues with reasonable fidelity (Lutolf et al., 2009). ECMs have also been isolated and extracted from various tissues, such as small intestine, skin (from cadavers), pancreas and breast (Rosso et al., 2005), and these ECMs have been used to engineer skin grafts (Badylak, 2007), enhance wound healing and to study tumor progression. One such example is given by porcine-derived small intestinal submucosa (SIS), which has proven clinical success for treating patients with hernias (Franklin et al., 2002) (reviewed in Badylak, 2007). Although these purified ECMs certainly have useful applications, their use is limited in scope owing to the need for well-defined microenvironments in tissue regeneration and stem cell transplantation in which animal byproducts and contaminants are limited. Moreover, to understand the molecular and biophysical mechanisms by which the ECM elicits diverse effects on cellular differentiation and morphogenesis it is crucial to use chemically and physically defined, modular ECMs that can be reliably reproduced. In this respect, synthetic

matrices have been developed that feature defined and tunable compositions, organization, mechanics and ECM remodeling capabilities. Indeed, in response to this need there has been literally an explosion of publications describing the generation and application of synthetic ECMs for tissue regeneration, and the reader is referred to some excellent reviews on these topics (Ayres et al., 2009; Dutta and Dutta, 2009; Lutolf and Hubbell, 2005; McCullen et al., 2009; Rosso et al., 2005; Zisch et al., 2003). One example is given by polyethylene glycol (PEG) hydrogels – frequently used biologically compatible synthetic matrices that support cell adhesion, viability and growth (Lutolf and Hubbell, 2005). Although these matrices can be covalently modified with ECM ligands and collagenase-degradable peptides and GFs (Ehrbar et al., 2007; Zisch et al., 2003), they do not mimic the organizational features of native collagen gels and all too often their pore sizes strongly impede cell migration. By contrast, peptide-based hydrogels, such as peptide-amphiphiles, assemble into secondary structures that recapitulate the collagen triple helix, and readily support stem cell growth and viability, and direct multicellular morphogenesis (Hauser and Zhang, 2010; Sieminski et al., 2008; Smith and Ma, 2004; Ulijn and Smith, 2008). These peptides-amphiphiles are amenable to modification by covalent binding of native proteins and MMP-degradable ECM peptides. Alternatively, poly(lactic-co-glycolic acid) (PLGA), a copolymer of glycolic acid and lactic acid (McCullen et al., 2009) that is inherently biodegradable as it is hydrolyzed into lactic acid and glycolic acid, has been developed and can be readily conjugated to various ECM ligands and peptides, or coated with collagen or chitosan to support cell adhesion, viability and growth. Indeed, one of the most exciting recent advances in the field has been the development of modular biocompatible ECMs, which contain ligand-binding cassettes and have tunable stiffness features that permit a precise patterning of cell adhesion in 2D and 3D formats (Serban and Prestwich, 2008). The realization that ECM organization is a crucial aspect of cellular behavior has led to the development of new methodologies and generated ECMs whose fiber size, orientation, stiffness, ligand-binding function and remodeling potential can be strictly controlled and monitored – including electrospun silk, and lactic-acid polymer (PLLA) and PLGA scaffolds (Zhang et al., 2009). Anisotropically nanofabricated substrates formed from scalable biocompatible PEG (Kim et al., 2010; Smith et al., 2009) are exciting new developments in the biomaterials field, whose only major impediment to their biological application appears to be a lack of

functional assessment in physiological culture assays and animal models. Although only time can tell whether this new generation of biomaterials will indeed prove useful, it is an appealing time to be an ECM biologist and our next challenge will be to embrace this smorgasbord of enticing new tools – which hopefully will at last allow us to decipher the language of the matrix.

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