Provisional matrix: A role for versican and hyaluronan

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Abstract

Hyaluronan and versican are extracellular matrix (ECM) components that are enriched in the provisional matrices that form during the early stages of development and disease. These two molecules interact to create pericellular "coats" and "open space" that facilitate cell sorting, proliferation, migration, and survival. Such complexes also impact the recruitment of leukocytes during development and in the early stages of disease. Once thought to be inert components of the ECM that help hold cells together, it is now quite clear that they play important roles in controlling cell phenotype, shaping tissue response to injury and maintaining tissue homeostasis. Conversion of hyaluronan-/versican-enriched provisional matrix to collagen-rich matrix is a "hallmark" of tissue fibrosis. Targeting the hyaluronan and versican content of provisional matrices in a variety of diseases including, cardiovascular disease and cancer, is becoming an attractive strategy for intervention.

Introduction

Throughout life, the extracellular matrix (ECM) constantly changes! ECM composition is controlled by the coordinate and differential regulation of synthesis and turnover of each of its individual components. These changes are driven by cytokines and growth factors and can involve the formation of matrices that are open and loose or compact and rigid. Disease often recapitulates development. Early in development, the ECM remodels to form a loose open network of molecules that facilitate cell division, cell movement, and cell sorting. While, in disease, the first stages of repair are the creation of an ECM that is open and loose, allowing cells to enter and repair tissue damage created by disease insult. Such an open and loosely organized ECM is, in both cases, referred to as a "Provisional Matrix".

Components of the ECM interact by entanglement, cross-linking, and charge-dependent interactions to form bioactive polymers which, in part, regulate the biomechanical properties of tissues and interact with cells to affect cell phenotype [1]. Usually, matrices that are soft and compliant are enriched in proteoglycans and hyaluronan, while matrices that are stiff and rigid are enriched in collagens and other fibrous proteins. The relative contributions of different ECM molecules vary with tissue type and result in mechanical and chemical properties appropriate to each tissue environment [2]. However, during development and in disease, the ECM undergoes changes to accommodate cellular events, such as proliferation and migration, and to maintain tissue homeostasis. Disturbances in the balance of these components create altered tissue architecture that impact tissue function. In fact, such ECM disturbances may determine or dictate the course of disease pathogenesis. Targeting the ECM is becoming an effective therapeutic strategy to treat human disease [3–5]. To understand the dynamic nature of the ECM and the functional consequences of ECM changes as tissues develop and remodel, classic wound healing is often given as an example of the transitions that occur [6–8]. Wound healing shares certain common features of ECM remodeling and with early events during embryonic development, such as in the development of the chick cornea or neural crest, the complete regeneration of a limb in amphibians [9–11], and in epithelial-to-mesenchymal transitions (EMT) [12].

With wounding, an ECM forms in a cell-free space, followed by the migration of mesenchymal cells into this ECM. These early changes involve the wound filling with a wave of cytokines and growth factors originating from broken or leaky blood vessels and cells entering and originating from within the damaged tissue. The early "provisional ECM" is formed by plasma proteins, such as fibrin, fibrinogen, and fibronectin, seeping into the wound site along with hyaluronan, either from the plasma or released from migrating mesenchymal cells. These components interact to form a crossed-linked gel which acts as a temporary scaffold for cellular events needed to repair the wound [6,8,11,13,14]. Thus, the provisional matrix serves as a scaffold and substrate for other cells entering into the wound bed, such as fibroblasts and smooth muscle cells. Such a substrate impacts the phenotype of the cells in contact with this matrix. For example, we showed that culturing arterial smooth muscle cells (ASMCs) on fibrin gels dramatically enriches these gels with decorin and biglycan, thus changing the mechanical properties of the fibrin gel. Such changes lead to alterations in the phenotype of the ASMCs [15].

In addition, during the early provisional phase of wound healing, inflammatory cells are drawn into the wound bed together with additional fibroblasts which further modify the composition of the provisional matrix. Such modifications include the synthesis and accumulation of hyaluronan and versican [16–18]. This is referred to by some as a "second order" of provisional ECM [19,20]. This review will consider the importance of hyaluronan and versican as specific ECM components of the provisional matrix and their role in regulating the phenotype of cells embedded in this matrix.

Hyaluronan and versican

Hyaluronan is a glycosaminoglycan (GAG) consisting of a linear polymer of repeating disaccharides of glucuronic acid and *N*-acetylglucosamine $[\beta(1,4)\text{-GlcUA}\beta(1,3)\text{-GlcNAc-}]_n$, and is synthesized by three different, but related, hyaluronan synthases, HAS1, HAS2, and HAS3 [21–24]. These are enzymes with multiple transmembrane domains and are situated on the inner surface of the plasma membrane of cells. During synthesis, the growing polymer is extruded through the membrane into the pericellular space. This is in contrast to the mode of synthesis of other GAGs, which are made and covalently linked to core proteins in the Golgi apparatus of the cell to form a proteoglycan, and then secreted by normal exocytotic mechanisms [25,26]. Hyaluronan chains can be anchored to the cell surface via the synthase enzyme or through binding to a cell surface receptor such as CD44 or RHAMM (receptor for hyaluronan-mediated motility). Hyaluronan is cleaved by one of several hyaluronidases. There are six hyaluronidase genes in humans, encoding enzymes with different properties and different cell locations [27,28]. Under normal physiological conditions, hyaluronan ranges in relative molecular mass from 10^4 to 10^7 Da (see reviews [29,30]). Hyaluronan can also self-associate to form fibers (cables), networks, and stacks. When retained at the cell surface, hyaluronan forms a voluminous pericellular matrix or "coat", which has also been termed "glycocalyx". The hyaluronan-dependent coat has multiple important roles, from serving structural and mechanochemical functions, to the regulation of cell division and motility [31]. High molecular weight hyaluronan (>500 kDa) has anti-inflammatory properties while low molecular weight (fragments < 500 kDa) promote inflammation [29,30]. Hyaluronan interacts with a number of cells including leukocytes, playing a critical role in immunity and inflammation [29,32,33]. Such interactions are prominent in diseases such as cancer and affect events that promote tumor formation such as progression and metastasis [34–39]. Hyaluronan is an integral component of the provisional matrix.

Versican is a large chondroitin sulfate (CS)-containing proteoglycan that interacts with hyaluronan through specific domains in its core protein [40]. Versican is synthesized by a variety of cells and in humans it is encoded from a single gene locus on chromosome 5q14.3 [41]. It is 86% identical between mouse and human [42], indicating the importance and highly conserved nature of this proteoglycan. Versican is encoded by 15 exons that are arrayed over 90 kb of continuous genomic DNA [43]. The central, GAG-bearing domain of the versican core protein is coded by two large exons, α-GAG and β-GAG, which can be alternately spliced with exon 7, which codes for the α-GAG region, and exon 8, which codes for the β-GAG region. When both the entire exons 7 and 8 are present and no splicing occurs, versican V0 is formed. When exon 7 is spliced out, versican V1 is formed. When exon 8 is spliced out, versican V2 is formed. When both exons 7 and 8 are spliced out, versican V3 is formed. This form of alternate splicing gives rise to versican variants that differ in the number of CS chains attached to the consensus sequence attachment sites in the core proteins (see reviews [43–45]). Since V3 does not contain any CS chains, it cannot be considered a proteoglycan, but it is frequently grouped with proteoglycans and characterized as such [46]. It is of interest that while V0, V1, and V3 are found in most tissues, V2 is mostly restricted to the central nervous system [47]. The CS GAG chains attached to the different isoforms of versican may differ in size and composition, depending upon the species, the tissue of origin, or the culture conditions that promote versican synthesis. For example, CS chains isolated from versican synthesized by ASMCs have a 6-sulfate: 4-sulfate ratio of 2 which increases to