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The role of hyaluronic acid and versican in the skin extracellular matrix

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Abstract

The extracellular matrix (ECM) is a structural network that comprises the bulk of the tissues. It acts as a supporting scaffold for the cells to develop their function and plays an active role in many processes such as proliferation and migration. Therefore, the ECM is an interesting object of study for regenerative medicine. In this article we make an extensive review of two key components of the ECM: the glycosaminoglycan Hyaluronic Acid (HA) and the proteoglycan Versican (Ver). These two molecules are present in the skin ECM and play active roles in processes such as differentiation, wound healing, hair follicle cycle and development. In our opinion, further biological insights in these two molecules and their interaction will be of great importance for skin tissue engineering applications.

Keywords*: Extracellular matrix, Glycosaminoglycans, Proteglycans, Hyaluronan, Versican*

Resumen

La matriz extracelular (ECM, del inglés Extra-Cellular Matrix), es un entramado estructural que compone el grueso de los tejidos biológicos. Actúa como un andamiaje para el desarrollo de las células, y juega un papel activo en varios procesos biológicos tales como en la proliferación celular y la migración. Por ello, la matriz extracelular es un interesante objeto de estudio para la medicina regenerativa. En este artículo, realizaremos un análisis de dos componentes clave en la matriz extracelular: el glicosaminoglicano Ácido Hialurónico (HA, por sus siglas en ingles Hyaluronic Acid) y el proteoglicano Versicano (Ver). Estas dos moléculas están presentes en la matriz de la piel y toman roles activos en procesos como la diferenciación celular, la reparación y la cicatrización de las heridas y el desarrollo del ciclo del pelo. En nuestra opinión, un entendimiento mas detallado en estas dos moléculas y las interacciones que entre ellas se producen probará ser de gran importancia para el campo de la ingeniería de tejidos dedicada a la piel.

Palabras clave: *Matriz extracelular, Glicosaminoglicanos, Proteoglicanos, Ácido Hialuronico, Versicano*

Accronym list

ECM – Extracellular Matrix	Col – Collagen
HA – Hyaluronic Acid	HAS – Hyaluronan Synthase
Ver – Versican	HYAL – Hyaluronidase
GAG – Glycosaminoglycan	HABR – Hyaluronic Acid Binding Region
PG - Proteoglycan	EGF – Epithelial Growth Factor
SC – Stratum Corneum	CBP – Complement Binding Protein

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Introduction

The extracellular matrix (ECM) is a structural network made up of proteins, saccharides and other components. The ECM acts as connective tissue, as well as a scaffold providing support to the cells. It was thought that the ECM was an inert, metabolically inactive substance and that its unique function was to provide structural support to the cells. However, this vision of the ECM as a passive component in cellular activity has been debunked.

The view of the ECM as a mere tridimensional (3D) scaffold was expanded with the discovery that growth of most human cells depends on cellular adhesion to the ECM through a mechanism called anchorage dependence. This mechanism allows cells to grow whenever they are anchored to a surface or a scaffold[1].

The ECM is known to play an active role in various cellular processes, such as proliferation, differentiation and migration[2,3]. It is formed by a variety of matrix macromolecules, being the composition and structure of these dependent on the tissue.

Components of the extracellular matrix

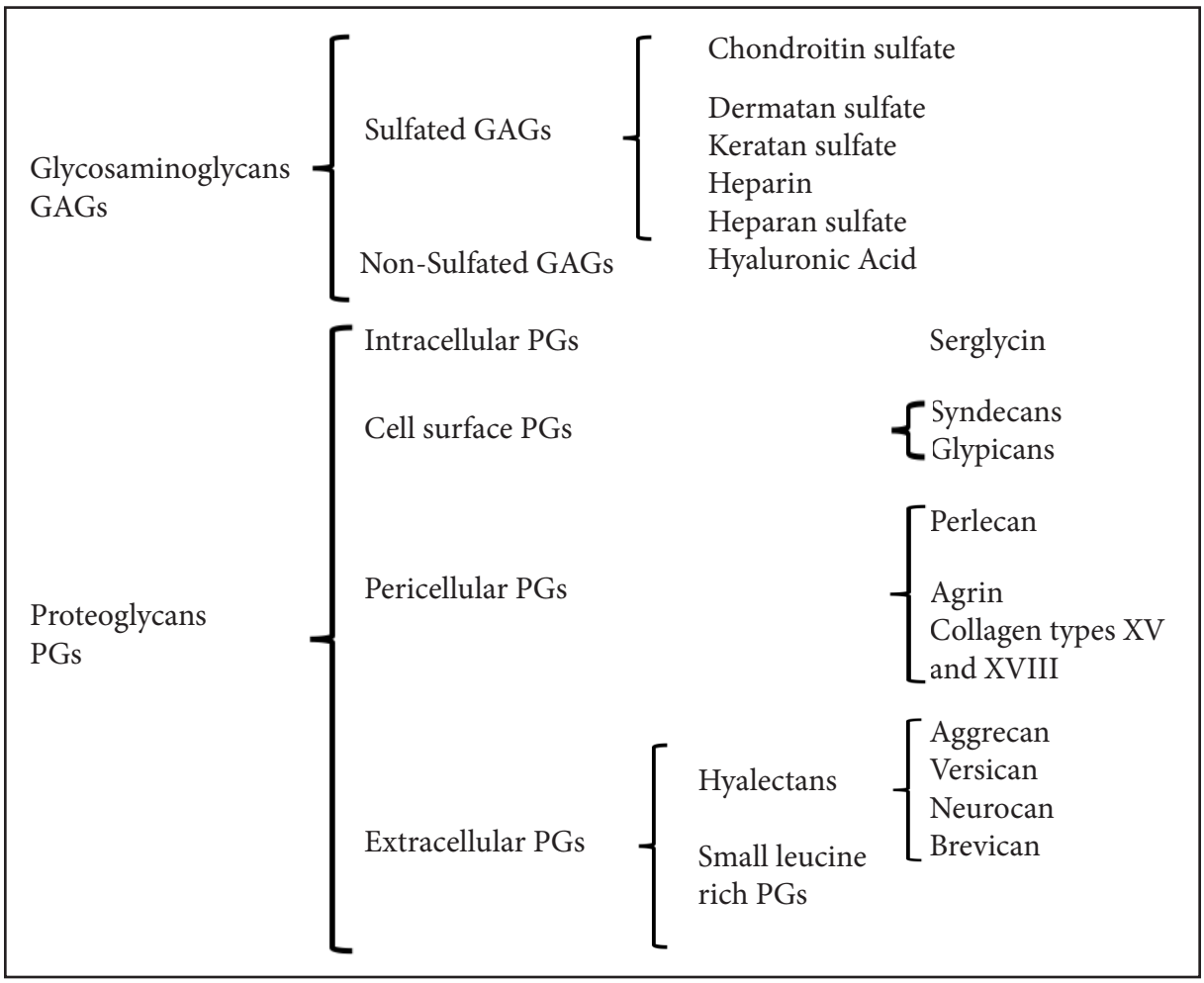
The main components of the ECM, regardless of the tissue, are fibrous proteins and proteoglycans (PGs)[4]. Fibrous proteins provide structural support to the ECM. They form three-dimensional structures that defines the mechanical properties of the tissues in the different organs. The most abundant fibrous protein in the dermal ECM is the collagen, which is in fact believed to be one of the most abundant proteins in the whole animal kingdom[5]. Other fiber-forming proteins present in the ECM include fibronectin, vitronectin an elastin[6,7].

Glycosaminoglycans (GAGs) are long, unbranched polysaccharide chains. They usually appear covalently bound to a protein core, forming proteoglycans. Glycosaminoglycans have a repeating disaccharide structure composed mainly of N-acetylated hexosamines (N-acetyl-D-galactosamine or N-acetyl-D-glucosamine) and D-/L-hexuronic acid (D-glucuronic acid or L-iduronic acid)[8]. GAGs are negatively-charged, which allows them to retain large amounts of water[9]. Since the length of GAG chains can vary widely, both among different GAG types and among chains of the same GAG type, the range of molecular weights in GAGs is very broad, ranging from a few kiloDaltons to over a hundred kiloDaltons. Glycosaminoglycans can be classified in sulfated and non-sulfated GAGs. Sulfated GAGs are chondroitin sulfate, dermatan sulfate, keratan sulfate, heparin and heparin sulfate, while hyaluronic acid or hyaluronan (HA) is the only non-sulfated gag[10]. GAGs interact with numerous growth factors, cytokines and chemokines[11], cell surface receptors and ECM molecules. GAGs participate in many cell functions, such as signaling, proliferation, migration, differentiation, apoptosis and adhesion.

Proteoglycans (PGs) can be classified depending on their localization into four families: intracellular, cell surface, pericellular and extracellular proteoglycans. Intracellular PGs are solely composed by Serglycin, which is the only characterized PG present in secretory compartments. Cell surface proteoglycans are formed by two main subfamilies of PG, which are syndecans and glypicans. Pericellular membrane PGs carry mostly Heparan Sulfate chains. As their name indicates they are present in the surface of many cell types that anchor themselves via cell surface receptors such as integrins. They are also part of basement membranes. This family is formed by perlecan, agrin and collagen types XV and XVIII. Finally, extracellular PGs form the largest class, which is composed by two subfamilies: hyalectans and small leucine-rich proteoglycans[12]. The family of the hyalectans is formed by the PGs aggrecan, versican (Ver), neurocan, and brevican, which share common structural features. Their N- terminal contains a HA-binding region, while the C-terminal domain binds to lectins.

Extracellular matrix of the skin

The skin is an organ composed by the following layers: epidermis, dermis and subcutaneous fatty tissue. Each of the layers have different cell populations and performs differential functions (Figure 1). Naturally, in each of the skin layers, the composition and structure of the ECM is different.



Scheme 1. Representation of the skin and the components of its ECM. The dermis, the bulk of the skin, has a rich ECM mainly composed by collagen, elastin and HA (images adapted with permission from Servier Medical Art freeware image bank).

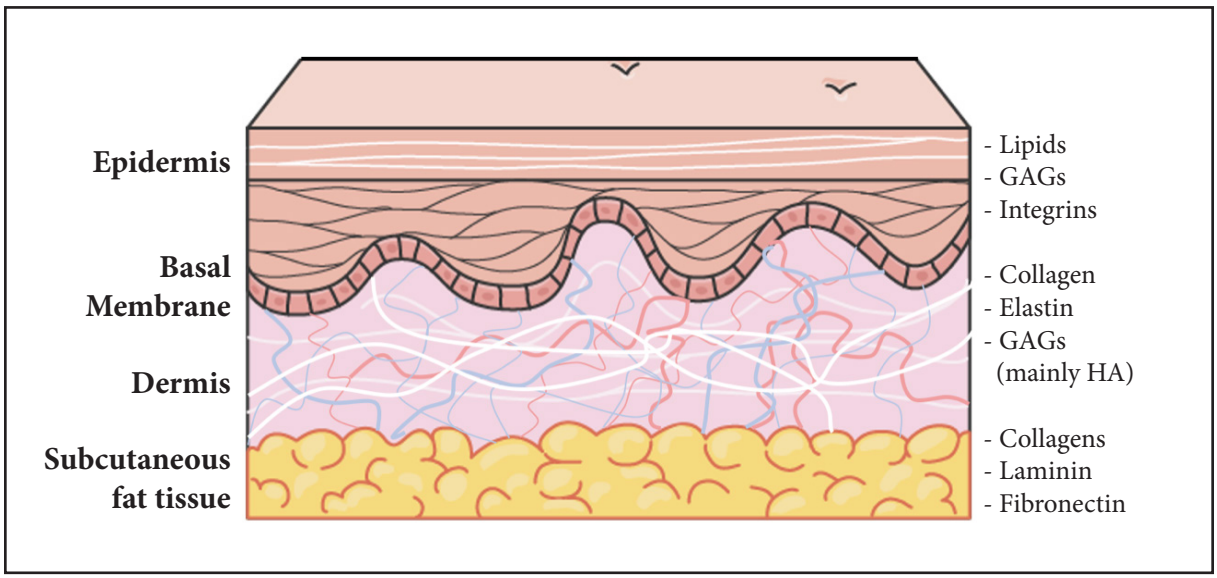


Figure 1. Representation of the skin and the components of its ECM. The dermis, the bulk of the skin, has a rich ECM mainly composed by collagen, elastin and HA (images adapted with permission from Servier Medical Art freeware image bank).

Epidermis. In the epidermis, the cells are highly packed. The keratinocytes and corneocytes (main cell population of the epidermis), are closely linked to each other through desmosomes[13]. Therefore, the spaces between cells are narrow, and the scarce ECM is constricted to these spaces. The outermost layer of the epidermis, the stratum corneum (SC) is where the cells are most packed, and the ECM, which is almost non-existent, is mainly composed by lipids. Ceramides, cholesterol, and non-essential fatty acids in the stratum corneum form lamellar layers, which composes its ECM[14]. As we move further down towards the basal membrane, the ECM becomes more abundant and includes proteins and GAGs such as HA or integrins[15]. Along the keratinocyte proliferating zone, intense Versican staining was observed[16].

Basal Membrane. Between the epidermis and the dermis, the basement membrane is placed. This porous membrane is semipermeable to lipids and keeps the other two layers together[17]. It is an acellular membrane, mainly composed by type IV collagen[18], to which cells from the lower portion of the epidermis attach.

Dermis. Below the basement membrane, the dermis can be found. The dermis comprises the bulk of the skin. Its ECM is much more abundant and significant than the epidermis ECM providing to the skin its mechanical properties. The main cell population are the fibroblasts. They are mesenchymal cells that play a key role in ECM formation through the secretion of growth factors and cytokines[19]. With respect to the ECM, it can be divided into fiber-forming structural molecules, nonfiber-forming structural molecules, and "matricellular proteins". Fiber-forming molecules form a three-dimensional structure that provides support to the ECM and defines the mechanical properties of the skin. The most abundant protein in the dermal ECM is collagen. Nonfiber-forming molecules function to create a charged, dynamic and osmotically active space, and are mainly PGs and GAGs. Amongst these, the most abundant is hyaluronan. Matricellular proteins are mainly present during wound healing, and act as dynamic signalling molecules1. Versican appears associated with the elastic fibers[16].

Subcutaneous fat tissue. The subcutaneous tissue is largely composed of fat cells and is often referred to as subcutaneous fat tissue or hypodermis. It is the layer between the dermis and the fascia. The function of the subcutaneous fat tissue is to provide thermal insulation, mechanical cushioning and store energy. Additionally, fat cells or adipocytes also have an endocrine function, secreting hormones such as leptin to alter energy turnover in the body and to regulate appetite. Adipocytes also have important signaling roles in osteogenesis and angiogenesis, and physical functions like phagocytosis. It has been reported that human fat contains multipotent stem cells[20].

The ECM of the subcutaneous fat tissue is mainly composed of collagens such as Col I, II, III, IV, V and VI, as well as subunits of Laminin and Fibronectin[21,22].

Relevance of HA-Ver relationship

As stated before, Versican is a proteoglycan from the aggregating chondroitin sulfate family. This family of proteoglycans have a tandem repeat in their G1 domains composed of two hyaluronan-binding domain repeats. This indicates a certain relationship between these two molecules. In fact, Versican colocalizes with hyaluronan in the pericellular matrix of cultured fibroblasts[23]. and in epidermal keratinocyte tumors. The presence of both molecules and their level of expression is a marker for the aggressiveness of the disease[24].

It is interesting to study the relationship between these two key ECM molecules as they are a perfect target for tissue engineering due to their relevant role in cell development. Other than the interesting properties of these molecules described in this review, as well as their interaction, it is appealing to study the co-expression of these two molecules in different ECMs. The findings would provide further insights in their relationship and their mechanisms in order to apply these two key molecules in skin tissue engineering. For example, while HA scaffolds are currently being investigated and developed, Ver is yet to be used in tissue engineering. Therefore, understanding these two molecules and their

interactions, would facilitate the preparation of biomimetic scaffolds with properties more similar to the ECM.

This manuscript is focused on the description of two essential components of the ECM, HA and Ver. In particular, their structures, their synthesis, their degradation, their location and the functions they perform on different tissues are described. Information about their interaction will also be reviewed.

Hyaluronic acid

Hyaluronan is an essential component of the ECM that acts as a space filler molecule that maintains hydration, serves as a substrate for assembly of proteoglycans and cellular locomotion, regulates cell proliferation and development and has a role in tumor progression[25-27], inflammation and wound healing[28,29]. It is a key structural molecule of the ECM, and as such, extensive studies have been carried out about its structure and function, although some aspect are still uncovered[30]. For example, it is well known that HA fragment size heavily affect the mechanisms for wound healing: high molecular weight HA promotes favors healing through promoting a fetal-like cell environment, whereas HA oligosaccharides favor fibrotic healing through stimulation of type I collagen. However, the mechanisms through which HA fragment size affect the ECM metabolism during wound repair require further investigation[31]. Other example that requires further investigation is the mechanism by which HA affects hair cycle[32].

Structure

HA is a GAG with a simple repeating disaccharide sequence: poly[(1à4)-β-D-glucopyranosyluronic acid-(1à3)-2-acetamido-2-deoxy-β-D-glucopyranosyl], with no evidence of heterogeneity or branching. It is the only glycosaminoglycan that is not covalently bound to a protein core. Regarding its size, in normal healthy tissue, it has a polydisperse distribution with an average mass of about 6000kDa, which corresponds to a chain length of about 15000nm, which occupies a spherical domain of about 600nm[33], referred to as high molecular weight HA (HMW-HA). In tissue under pathological conditions such as cancer, inflammation or tissue remodeling, the HMW-HA is degraded faster, giving place to a higher concentration of low-molecular weight HA (LMW-HA). LMW-HA can be further degraded to shorter oligomers (o-HA). The size of HA molecules plays a role in cell signaling[34,35].

HA exists in solution in a flexible, coiled configuration, which allows HA to entrap large amounts of water. In dilution, HA coils start to entangle at concentrations lower than 1mg/mL. For large size molecules (larger than 6000kDa), this entanglement starts at the concentration at which chains would fill the solution and be forced to overlap domains, at 320μg/mL. This overlapping of the domains

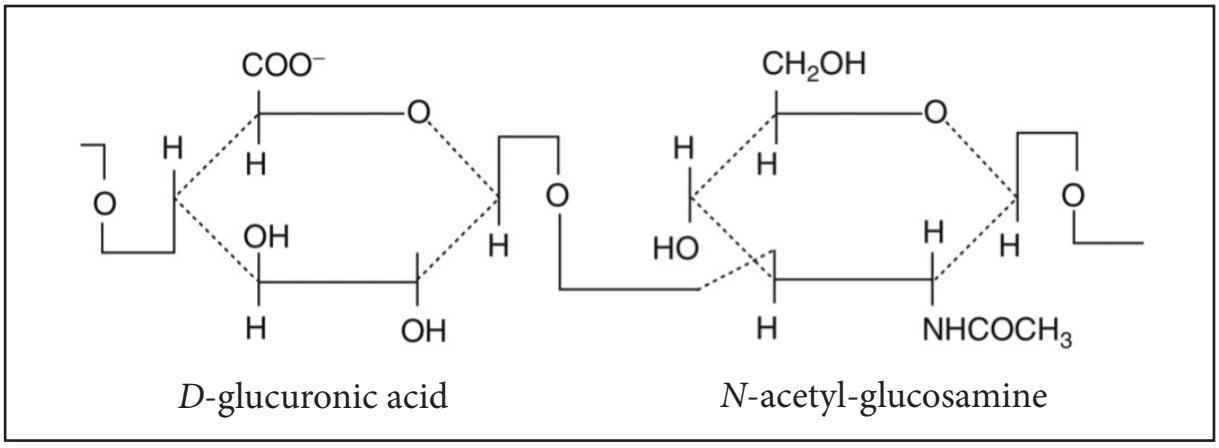


Figure 2. Hyaluronic acid structure:
(1→4)-β-D-glucopyranosyluronic acid-(1→3)-2-acetamido-2-deoxy-β- D-glucopyranosyl[33].

causes HA viscosity to separate from the ideal HA concentration. If mutual macromolecular crowding (domain overlapping) were absent, the ideal behavior would be followed. In a real polymer solution, the nonideality contribution due to crowding causes a nearly exponential increase in specific viscosity as the overlap between polymer domain increases[33].

Synthesis

Apart from being the only non-sulfated GAG, and the only GAG that is not found covalently bound to a protein core, HA is also the only GAG that is not synthesized in the Golgi apparatus. In vertebrates, HA is produced on the inner face of the fibroblast plasma membrane and directly extruded onto the ECM by a family of transmembrane proteins called Hyaluronan Synthases (HAS1,2,3). The differences between the three proteins are not clear, although it has been reported that they produce HA chains of different lengths[36,37].

Degradation

HA has been demonstrated to have a high turnover rate[39-41]. It has a half-life of 3 to 5 minutes in the blood stream and less than a day in the skin, being the total turnover in humans of around 15 grams per day. In the skin, about 20-30% percent of the turnover occurs by metabolic degradation in situ, while the rest is removed by the lymphatic system and subsequently degraded in the lymph nodes and liver. The elimination of HA through metabolic pathways occurs by the degradation of this GAG into fragments of varying size. This is achieved by hydrolysis with the hyaluronidases enzymes (HYAL) of which six different homologous genes have been found[42-44]. The two primary mammalian hyaluronidases, Hyal-1 and Hyal-2, have different activities and degrade HA to either oligosaccharides or larger fragments. Several HA-degrading enzymes can be found in bacteria, fungi, and other organisms such as leeches[45].

Degradation of HA can also occur due to thermal and chemical causes. Thermal degradation of HA has been proven to occur. HA degradation at 37°C occurs at a higher rate (measured as weight- average molecular weight reduction) for lower molecular weight fragments of HA. Exposure to higher temperatures also affects thermal degradation of HA. At 60°C, degradation is similar to physiological temperature, whereas at 90°C, degradation is severely increased even in short periods of time[46].

Regarding chemical stability of HA, hydrolysis of HA was measured for different pH values. The degradation process is random and follows first-order kinetics independently of the pH value. However, lower pH values account for a higher degradation rate of HA. A linear dependence of the hydrolysis rate of HA on the proton concentration is expected. However, a change in the slope is observed for HA around pH 3-4 around the apparent pKa value of the GlcA unit of HA[47].

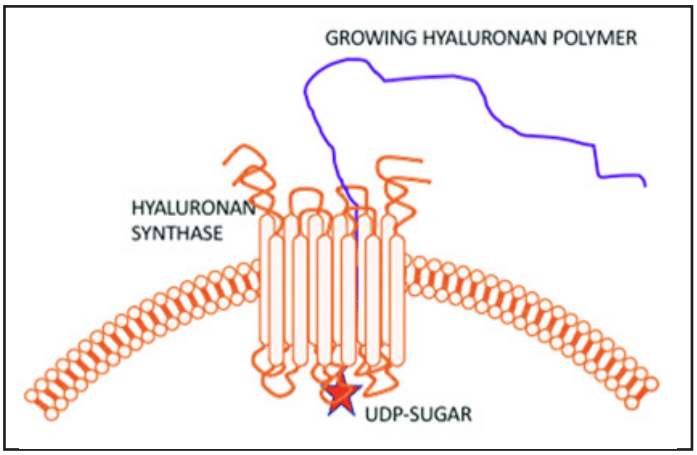


Figure 3. Schematic structure of the transmembrane protein: the hyaluronan synthase.[38](Image modified from McCarthy et al., licensed by CC BY 4.0).

Location and distribution

An exhaustive analysis of the HA content in the body of the rat was performed by Reed et al. in 1988. The results obtained from this analysis should be applicable to other mammals, due to the similar functions carried out by this glycosaminoglycan in all mammals species. More than half of the whole hyaluronan in the rat body was retrieved from the skin. About a quarter of HA was found to be located in the bones and joints taken together. Less than 10% was found in the muscles, while other organs contained only minor amounts of the GAG[48].

The distribution of HA within a tissue is not homogeneous either. Using histological techniques, it has been reported that, for example, in the skin, the content of HA in the dermis is much higher than that of the epidermis. Furthermore, in the dermis, the papillary dermis has a much higher HA content than the reticular dermis[49].

Roles

HA plays important roles in several processes such as skin hydration, tissue repair, angiogenesis and tumor progression.

Hydration. Due to its hydrophilic nature and its ability to entrap large amounts of water, HA plays a key role in skin hydration. HA is more abundant in the dermis than in the epidermis, but it plays a key role in hydration of the epidermis. The epidermis is separated from the dermis by the basement membrane and it is not vascularized. Therefore, the hydration of the epidermis depends on the HA-bound water[50]. Skin aging is characterized by the disappearance of HA in the epidermis. This means the loss of the main molecule responsible for hydration in the epidermis. Thus, skin moisture is reduced. The mechanisms for the loss of HA in the epidermis with aging are still unknown[51,52].

Tissue repair. HA plays a role in the inflammatory response in wound healing. HA fragments produced during inflammation induce a collection of genes in macrophages, including several members of the chemokine gene family[53]. Furthermore, CD44 (the HA cellular receptor) plays a key role on leukocytes as a receptor for transendothelial migration at sites of inflammation. In this way, discrete populations of cells in peripheral blood are directed to migrate at inflammatory sites, where they recognize HA displayed on the surface of activated endothelial cells[54,55].

Angiogenesis. The correct formation of new blood vessels is an essential process for wound healing and repair of damaged tissues. However, excessive angiogenesis can be detrimental, since it may promote tumor growth and metastasis. GAGs, and notably HA, has been reported to play a key role in angiogenesis. Native high molecular weight HA is anti-angiogenic, while the products of degradation of HA (3-10 disaccharides) stimulate endothelial cell proliferation, migration and tube formation by activation of specific HA receptors CD44 and Receptor for HA-Mediated Motility (RHAMM, CD168)[56].

Tumor progression. HA plays a dual role in tumor progression. High molecular-weight

	Weight (g)	Total HA (mg)	HA (%)
Whole rat	201	60.5	100
Skin	40.2	33.8	56
Muscles	35.7	4.69	8
Skeleton and supporting tissues	57.6	16.2	27
Intestines and stomach	15.8	0.50	1
Remaining internal organs	43.4	5.25	9

Table 1. Content of HA in the organs of the body48. More than half of the total content is found in the skin (56%), while around a quarter (27%) in the skeleton and supporting tissues.

HA is found in normal tissues and maintains homeostasis and restrains cell proliferation. During processes such as tissue remodeling and wound healing, HA is fragmented into low molecular weight polymers, which promote inflammation, immune cell recruitment and angiogenesis[44-47]. Tumor cells hijack the tightly regulated HA production/fragmentation. The processes associated with wound repair and remodeling then participate in driving and maintaining malignant progression. Moreover, elevated high molecular-weight HA production is related with cancer resistance[25].

Versican

Versican (Ver) is a member of the aggregating chondroitin sulfate proteoglycan family, which also includes aggrecan, neurocan and brevican[16,57]. However, unlike aggrecan, which is almost exclusively expressed in cartilage and brain, and neurocan and brevican, which are proteoglycans expressed in the central nervous system, Ver is expressed in the ECM of a variety of tissues and organs[58].

Structure

The size of Ver varies depending on its isoform. It is formed by 3 structural domains: N-terminal domain or G1, C-terminal domain or G3, and a chondroitin sulfate chain binding region between G1 and G3 (Figure 4). The G1 domain is composed of an immunoglobular (Ig)-like motif, followed by two proteoglycan tandem repeats known as HA-binding region (HABR). The G3 domain consists of two epidermal growth factor (EGF)-like repeats, a carbohydrate recognition Lecitin-like domain and a complement binding protein (CBP)-like motif [59,60]. G1 and G3 domains are common to the whole aggregating chondroitin sulfate proteoglycans. Regarding the chondroitin sulfate binding region, it is formed by two subdomains, α-GAG and ß-GAG. Four isoforms of Ver exist as a combination of the domains. V0 occurs when both α-GAG and ß-GAG in the chondroitin sulfate binding region are present. V1 and V2 occur when only the ß-GAG or the α-GAG subdomain is present, respectively. V3 occurs when neither α-GAG nor ß-GAG subdomains are present[61].

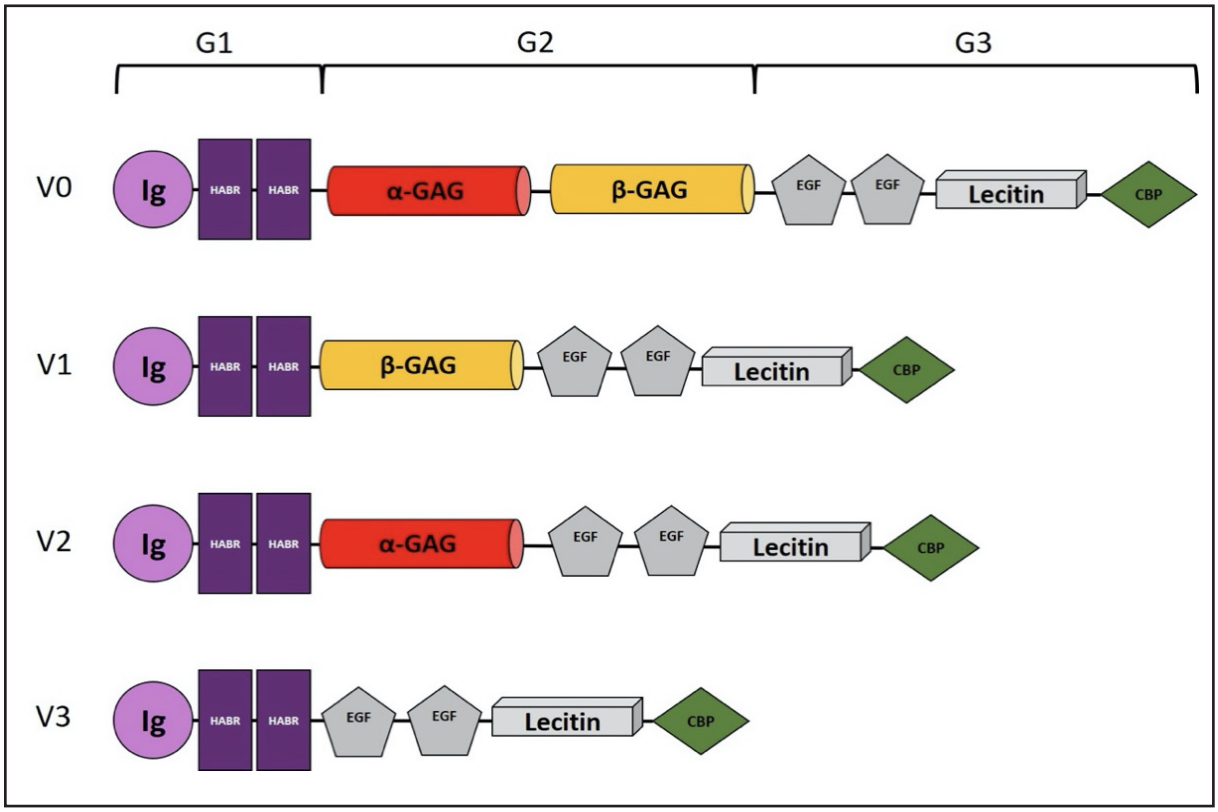


Figure 4. Versican and its isoforms resulting from alternative splicing. The exons that are the product to this process of alternative splicing are those coding for the chondroitin sulfate-binding chain (α-GAG and ß-GAG domains).

Synthesis

Versican is coded by the VCAN gene in chromosome 5q13.2[62] in humans divided into 15 exons over 90-100kb[63]. The existence of different isoforms of Versican is due to the process of alternative splicing, namely, of exons 7 and 8, coding for the α-GAG and ß-GAG subdomains, respectively. When both the entire exons 7 and 8 are present and no splicing occurs, V0 is formed. When exon 7 is spliced out, V1 is formed. When exon 8 is spliced out, V1 is formed. Finally, when both exon 7 and exon 8 are spliced out, V4 is formed[58].

Degradation

Several proteinase families are capable of degrading Ver. For example, matrix metalloproteinase (MMP)-1,-2,-3,-7, and -9 have been shown to degrade Versican in vitro[64-66]. Plasmin has been shown to degrade Versican as well67. Finally, ADAMTS (a desintegrin and metalloproteinase with thrombospondin motifs)-1, -4,-5 and -9 have been shown to cleave versican[68-71].

Distribution

Versican is largely distributed along the whole body being part of the ECM in several tissues. Versican performs different roles. For example, it has modulatory roles in cell adhesion, migration and proliferation[72-74]. In vitro experiments have demonstrated that regulates the attachment of cells to various extracellular matrix components such as collagen I, fibronectin and laminin. There are indications that it may also participate in the control of keratinocyte and dermal fibroblast proliferation. A thorough study using immunohistochemistry techniques revealed the distribution of Versican within different tissues of the human body[16].

Roles

As stated before, Ver has 4 isoforms each with a give structure resulting from the process of alternative splicing. Each isoform may play different roles in a given tissue process. In this section, we focus on the most relevant functions played by Versican in the skin.

Hair development and cycling. Hair development is described as a cycle of three phases: anagen (or growing phase), catagen (or transition phase) and telogen (or resting phase) [75]. The expression of Ver in the dermal papilla is more abundant in the anagen phase, diminishing in the catagen phase and being almost nonexistent in the telogen phase. However, in telogen follicles, Ver expression is found in the neck region of the hair. As hair cycle begins again, Ver disappears from the neck region and is again prominent in the dermal papilla. The expression of Ver in the growing phase of the hair dermal papilla indicates that it plays a significant role in hair cycle and hair development [76]. Furthermore, the expression of Ver is almost lost in the dermis of vellus-like follicles affected by male pattern baldness[77].

Provisional matrix. Hyaluronan and Versican interact to create pericellular "coats" and "open space" that facilitate cell sorting, proliferation, migration, and survival. They also contribute to the recruitment of leukocytes during development and in the early stages of disease. Those molecular complexes play important roles in controlling cell phenotype, shaping tissue response to injury and maintaining tissue homeostasis. Conversion of hyaluronan-/Ver-enriched provisional matrix to collagen-rich matrix is a "hallmark" of tissue fibrosis. Targeting the hyaluronan and Versican content of provisional matrices is becoming an attractive strategy for intervention in a variety of diseases including, cardiovascular pathologies and cancer[78].

Proliferation and apoptosis. Versican isoforms V1 and V2 have been shown to play important roles in cell proliferation and apoptosis, respectively. A study in which a mutant isoform of Ver V1 possessing a 2367-base pair deletion in its ß-GAG domain abolished V1's proliferative activity, proved that ß-GAG domain in V1 is essential for its promoting effect in cell proliferation. Similarly, a mutant isoform of V2 with a 1953-base pair deletion in the α-GAG domain lacked the inhibitory activity of V2, hence suggesting that α-GAG is essential for V2's inhibitory activity[74].

Tissue	Versican distribution
Cardiovascular system	Ver-positive capillaries between the Ver-negative heart muscle cells. In large blood vessels, all three layers presented staining, while in normal muscular arteries, Ver is restricted to the adventitia.
Lymphatic organs	Intense Ver staining was confined to the connective tissue of the capsule and around central arterioles in the spleen.
Supporting tissue	In tendon, no Ver staining was observed other than in positive foci probably originated from fibrocytes and the capillary network. Ver staining is not present in the cartilages (neither in the ECM nor in the cells) of the ribs and the trachea. While both the ECM and the cartilage tissue in the epiglottis and in the intervertebral discs are positive. No staining was found in association with skeletal muscle fibers. The endomysium of the intrafusal fibers of the muscle spindle showed intense staining.
Skin and breast tissue	Intense Ver staining is observed along the keratinocyte proliferation zone in the basal cell layer of the epidermis, including the sheaths of hair follicles. In the dermis, Ver appears associated with elastic fibers. In the breast, connective tissue shows weak staining. No staining is found in the epithelial component of this tissue.
Gastrointestinal tract	Ver was detected in the smooth muscle tissue of the esophagus, as well as in the sent in the muscular layers of the intestine. In the liver, staining was observed in the portal tracts. In the pancreas, Ver is localized in the connective tissue surrounding the exocrine acini and in various ducts and blood vessels.
Respiratory tract	Intense staining is shown in the subepithelial connective tissue. In the alveolar walls, Ver was co-localized with the fine fibers of the elastic network.
Endocrine organs	The connective tissue surrounding the adrenal cortex showed Ver staining. In the subcapsular region and the zona glomerulosa, staining intensity was increased. In the thyroid, weak staining was observed at the luminal surface of thyreocytes and in the subepithelial connective tissue. In the adenohypophysis, only the network of connective tissue and capillaries surrounding nests and cords of endocrine cells contained Ver.
Urinary tract	In the bladder and the ureter, layers of connective tissue and smooth muscle cells stained positive, covered by Ver-negative transitional epithelium. In the kidney, Ver was present in the surrounding connective tissue, with more pronounced staining around regressively transformed glomeruli.
Female genital tract	In the ovary, Ver was present in the large blood vessels of the medulla. A strong immunoreaction was observed in the dense plexus of elastic fibers beneath the epithelium of the vagina. In the placenta, Ver was localized in the villous mesenchyme and around blood vessels.
Male genital tract	In the testis, Ver was restricted to the tunica propria surrounding seminiferous tubules. In the prostate, a strong linear Ver immunoreaction was observed at the apical surface of the columnar cells of the epithelium.
Peripheral and central nervous system	In the peripheral nervous system, nerves were surrounded by Ver-positive perineurium. In the central nervous system, staining of white matter similarly to glial fibrillary acdic protein suggests a close association of Ver expression with astrocytes.

Table 2. Distribution of the proteoglycan Ver in the human body16.

Tissue engineering background for HA and Versican

Hyaluronan has been widely used in the field of tissue engineering, in particular, in cartilage and skin regeneration. Some studies propose to apply an injection of hyaluronan under the skin to promote its regeneration. It can both be used as a carrier matrix containing (diverse components, such as human cells or growth factors[79]) or as unique component,has been proven to stimulate collagen synthesis in damaged human skin[80]. Other studies indicate that HA can be used as a drug delivery agent for various routes of administration such as ophthalmic, nasal or parental, and more recently, as a topic drug delivery agent[81]. However, the main use of HA in skin engineering is as a scaffold for wound healing and tissue repair[82-86]., The main advantages of the use of HA as a scaffold in tissue engineering are: 1) HA is involved in skin wound healing and angiogenesis [87].Therefore, is a promising candidate for skin scaffolding for tissue engineering and repair; 2) it is also hydrophilic, non-adhesive and biodegradable, which means that HA scaffolds degrade rapidly in aqueous solution and it is eligible for use in vivo; 3) HA contains functional groups that can be used to produce hydrogels by chemical crosslinking or introduce functional domains to achieve tuneable biological and mechanical properties in the scaffold; 4) furthermore, due to its ability to retain water, HA scaffolds are able to maintain an hydrated environment, ideal for wound grafts85.

Unfortunately, there exists a number of disadvantages that are yet to be overcome. Most of them arise from the production process. Historically, HA has been obtained from many animal sources, mainly rooster comb. While HA itself is non-immunogenic, other matrix proteins can be mixed with the HA solution. Consequently, many purification steps are needed to provide a safe solution of HA. Recently, many companies are offering HA secreted by microorganisms such as certain strains of Streptococcus zooepidemicus or S. equi. However, the risk of mutation of the bacterial strains and possible co-production of toxins, pyrogens or immunogens are an important concern with fermentative HA. Therefore, HA from rooster comb is still currently preferred for human treatment[88]. Furthermore, even if purity of HA is not required, it is not desirable to provide samples with a heterogeneous distribution of HA molecular weight. Low polydispersity is essential as HA would infer different processes depending on its molecular weight[89].

Regarding the use of HA as a scaffold, the main problem arises from its mechanical properties. HA solutions do not form scaffolds with long lasting mechanical integrity. To achieve tailored mechanical properties and degradation rates, chemical modification and crosslinking of HA is often necessary. However, this is not an ideal solution, since HA in the ECM does not appear as a crosslinked matrix, and therefore, is not an ideal environment for cells[90].

Versican has not been as widely used as HA in tissue engineering of the skin. Nevertheless, it has been used as a marker for skin health. Some studies have been carried out on its role as a factor promoting skin development[91]. The main interest of Versican in skin engineering is due to its role in hair follicle development and cycling, being hair production an emerging field of research[92].

Conclusions and future perspectives

As presented throughout this review, HA and Versican are two very appealing molecules to study due to the important role they play in several physiological processes such as differentiation and wound healing. Nowadays, they are mainly targeted for cancer therapies, since they take part in tumor progression[93,94]. Tissue engineering could also make use of HA and Versican of facilitate tissue regeneration. They could be added to scaffolds to imitate the native ECM with the idea of replicating in vitro the processes that cells undergo in this natural scaffold. To be able to create realistic ECMs in vitro, further studies are needed to characterize the distribution and interaction of these two molecules. As future work, a study will be carried out in which, using immunohistoche-

mistry and immunofluorescence, the location, the distribution and the co-localization of these two molecules will be analyzed.

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