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GLYCOSAMINOGLYCANS EVOLUTION: FROM "INERT GLUE" TO MASTER REGULATORS OF SKIN BIOLOGY

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Glycosaminoglycans (GAGs) are the most abundant heteropolysaccharide in the body. Their research started almost a century ago and since then, they experienced a remarkable evolution transitioning from "inert glue" to moisture-binding components to finally being recognized as one of the blocks of life and master regulators of cell biology. The late entrance of GAGs in cutaneous research is due to a combination of their complex biology and the central paradigm of the 20th century molecular biology research: "DNA to RNA to protein." With the new millennium, new methodologies for GAG analysis and separation were developed elucidating GAG synthesis and degradation pathways and the role of post-assembling modifications in their activity. Today, GAG research constitutes one of the most exciting and challenging areas of modern medicine as these dynamic and multi-functional molecules interact with hundreds of partner proteins regulating their availability and function under physiological and pathological conditions.

GAGS 101: STRUCTURE, FUNCTION AND BEYOND

Carbohydrates can be divided into simple sugars and complex conjugates also known as glycans. GAGs are large, linear, negatively charged glycans. Among the four classes of GAGs known today, namely: chondroitin sulfate (CS)/dermatan sulfate (DS), heparin/heparan sulfate (HS), keratin sulfate (KS) and hyaluronic acid (HA), only the latest is non-sulfated nor forms part of proteoglycans. GAGs are present on all animal cell surfaces or as components of the extracellular matrix (ECM) and are required for cell viability. Biosynthesis of GAGs is an energy-intensive process. GAG's half-lives are relatively short (days) in comparison with other molecules present in the skin (collagen 15 years and elastin 75 years), resulting in a significant cellular effort (energy and time) to preserve their homeostasis. GAGs display a rather simple basic structure formed by disaccharide repeating regions (uronic acid [D-glucuronic

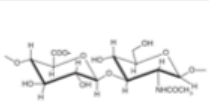
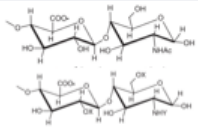
	Hyaluronic acid (HA)	Heparan sulfate (HS)
		
Sulfated	NO	YES
Covalently Attached to Proteins	NO	YES
Site of Synthesis	Plasma membrane	Golgi apparatus
Major Turnover Regulators	Synthesis: hyaluronic acid synthase Degradation: hyaluronidase	Synthesis: Heparan sulfate synthase. N-deacetylase/N-sulfotransferase. 2, 3 and 6-O sulfotransferases. C-5 epimerase. Degradation: heparanases
Tissue Distribution	ECM of connective tissue	Cell surface, basement membranes and ECM

Table 1: Comparison of key parameters between hyaluronic acid and heparan sulfate, a SuGAG

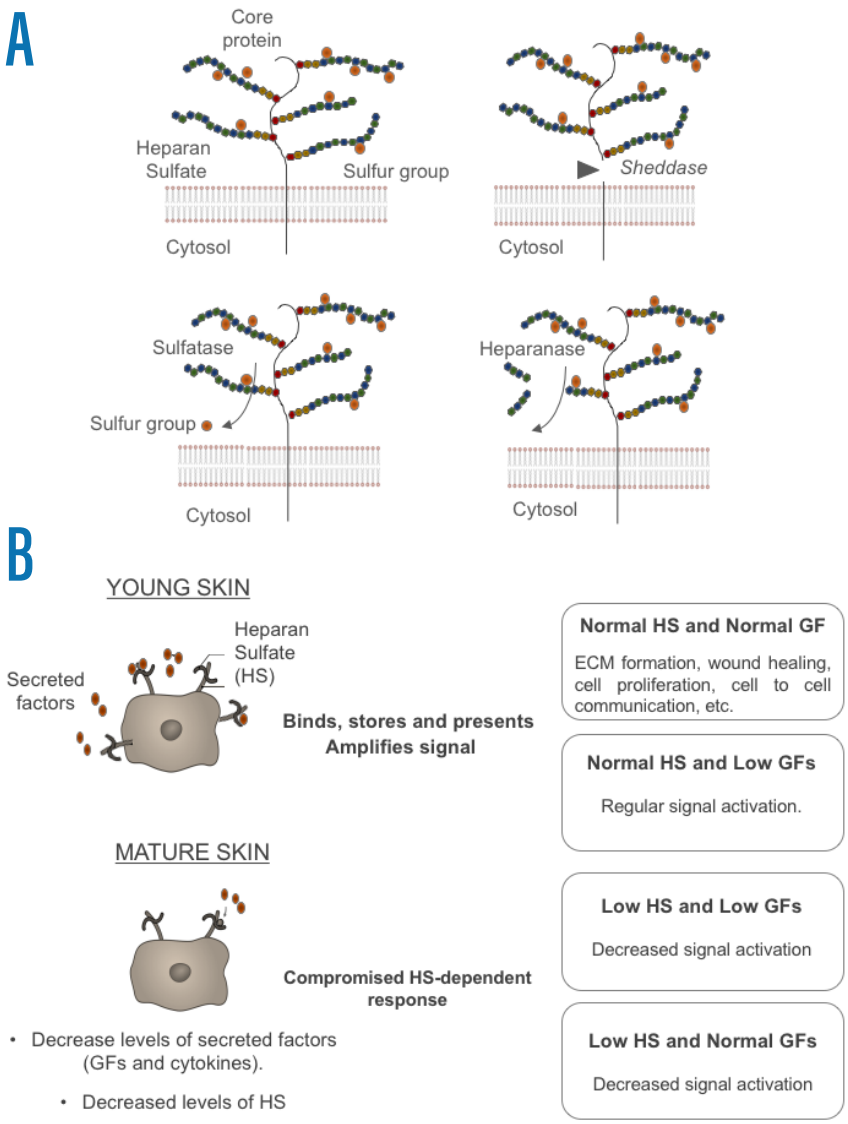


Figure 1: (A) Extracellular modifications of HS result in changes in its activity. Sheddase: releases HS from the cell surface. Sulfatase (6-O endosulfatase): removes HS sulfation decreasing GF signal. Heparanase: produces biologically active fragments. (B) Secreted factors signaling depends on endogenous HS levels. As we age, levels of secreted factors as well as endogenous HS diminish resulting in decreased signal activation.

acid or L-iduronic acid] and amino sugar [D-galactosamine or D-glucosamine]). The GAG sugar backbone can be sulfated in various positions creating a great variety of potential modifications (million options by octasaccharide sequence).[1] Sulfation triggers conformational changes modifying interactions with partner proteins. Metal ions and changes in pH also modify GAG-protein interactions.

GAGs bind water and they have been largely used, HA mainly, in the cosmetic industry as moisturizers and dermal fillers. GAG-water binding capacity provides resistance to compressive forces. Water molecules can surround or be squeezed out from GAG's surface changing the volume occupied. Once that compression is removed, GAGs are able to regain their hydrated volume due to the repulsion arising from their negative charges. GAGs also hydrate pre-existent collagen and elastic fibers improving their functionality and resistance. In addition, GAGs are biologically active molecules that regulate numerous pathways involved in tissue developing, remodeling and healing. This characteristic is strongly linked to their interaction with target proteins and is influenced by degree and pattern of sulfation and composition and structure of the carbohydrate bone. The clinical significance of sulfated glycosaminoglycans (SuGAGs) is evidenced by the numerous inherited disorders that are associated to defects in genes of sulfate transport membranes, activated sulfur synthesis, or sulfotransferases. HS is one of the most extensively studied SuGAGs. In the skin, endogenous HS plays a critical role in skin health, defense, wound healing, aging, atopic dermatitis, rosacea and psoriasis.

ENDOGENOUS HEPARAN SULFATE (HS)

HS is a vital component of all animal cells.[2,3] Its key role derives from its interactions with a plethora of partner molecules including growth factors (GFs), chemokines-cytokines, ECM components, enzymes, morphogens and pathogen derived molecules. HS also facilitates self-assembly

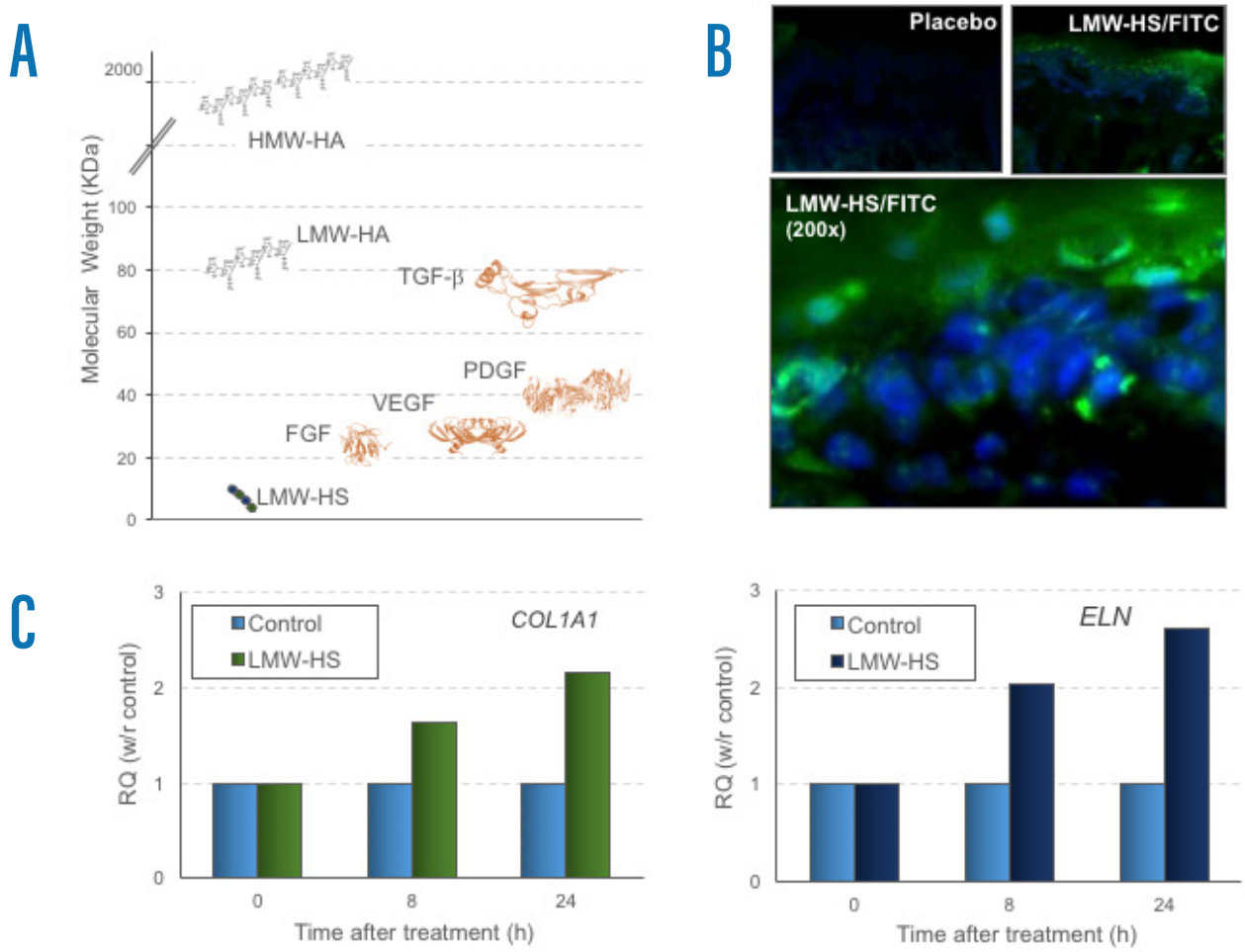


Figure 2: Low Molecular Weight Heparan Sulfate (LMW-HS) or HSA. (A) Size comparison between different molecules and LMW-HS. HMW-HA (high molecular weight hyaluronic acid), LMW-HA (low molecular weight hyaluronic acid), TGF-β (transforming growth factor), PDGF (platelet derived growth factor), VEGF (vascular endothelial growth factor) and FGF (fibroblast growth factor). (B) Penetration of LMW-HS into human skin. Skin explants were treated twice (every 12 h) with a cream containing LMW-HS (0.5%) conjugated with FITC (green). Placebo group was treated only with the vehicle. Nuclei were stained with DAPI (blue) (C) Collagen and elastin induction by LMW-HS (1%). Gene expression was evaluated by qRT-PCR. Samples were harvested 8 or 24 h after treatment. *COL1A1*: collagen type 1 and *ELN*: elastin. Values over 2.0 are consider significant with respect to the control.

and structural integrity of ECM components. Synthesis of HS occurs in the Golgi apparatus where the core structure is heavily modified by epimerases and sulfotransferases (Table 1) yielding heterogeneous structures. Interestingly, relatively short HS chains (40 disaccharides or so) can hold millions of distinct structural possibilities, explaining why HS has been called "the most information-dense biopolymer in nature."[4]

Extracellular HS activity can be further modified by the action of three enzymes: sheddase, 6-O endosulfatase and heparanase (Figure 1A). The expression of these enzymes is tightly regulated and results in boosting or inhibition of secreted factors signaling. As mentioned above, HS interacts with a large number of GFs such as fibroblast growth factor (FGF-1, 2 and 4), vascular endothelial growth factor (VEGF), and

heparin-binding epidermal growth factor (HB-EGF). HS binds, stores and presents these GFs (Fig. 1B). GF signaling often requires interactions with HS and therefore, decreased levels of HS reduces cell response to GFs regardless of the abundancy of these secreted proteins (Fig. 1B). On the contrary, decreased levels of GFs can trigger physiological responses in the presence of HS due to its capacity to amplify cell signaling (decreases threshold of receptor activation). This unique characteristic plays an important role in skin conditions associated with diminished GF levels such as aging.

LOW MOLECULAR WEIGHT HEPARAN SULFATE (LMW-HS) AND AGED SKIN

For years the skin aging dogma had been to attribute this process to the loss of mature collagen due to a combination of increased proteolytic activity and decreased synthesis. However, strategies focusing only on collagen improvements remain unsatisfactory suggesting that other factors play equal or more important roles in the development of senescence. Chronological and environmental aging trigger intense dermal and epidermal GAG remodeling. Sun-exposed skin is dehydrated and shows changes in the ratio of abundancy of SuGAGs, which trigger solar elastosis.[5] Deposit of elastotic material not only affects the dynamics of the ECM but also increases SuGAG-linked dysfunction by sequestrating these molecules and compromising both their biological activity and water trapping capacity. Skin aging also is characterized by diminished cell-cell communication, cell adhesion, cell-proliferation, formation, assembling and integrity of ECM components and wound healing responses. Interestingly, all these functions are modulated by SuGAG, more specifically HS, which levels decreased within aging significantly affecting skin homeostasis.

There are two major caveats to the utilization of HS in topical skin products:

1.) HS skin penetration is limited by its large molecular size, highly polar charge and shape and

2.) HS multi-functionality, which strongly depends on the diverse post-assembling modifications (i.e, sulfation), it is only accomplished *in vivo*. Low Molecular Weight Heparan Sulfate (LMW-HS) or HSA (Heparan Sulfate Analog) was developed to overcome these two obstacles. LMW-HS is a modified form of naturally produced HS in which size, shape and charge were optimized to ensure skin penetration. This molecule is smaller than low molecular weight hyaluronic acid (LMW-HA) and several GFs commonly used in the cosmetic industry (Figure 2A). *Ex vivo* studies showed that LMW-HS not only penetrates human skin (Figure 2B) but remains biologically active, as demonstrated by the induction of collagen (peaks at 24h) and elastin (peaks at 8h) in a skin model (Figure 2C).

FINAL REMARKS

During the last years, a huge leap in the field of GAGs has been accomplished, positioning them as essential players in tissue development and remodeling, homeostasis, and disease progression. Glycan research, more specifically SuGAGs, has opened up a new approach to study and treat aging as these multifunctional molecules are able to address four major signs of skin aging: sagginess, wrinkles, dehydration and uneven pigmentation. Technical difficulties to deliver these large and highly polar molecules to the skin needed to be addressed to provide the anti-aging benefits described above. An example of this approach is the bio-engineering of LMW-HS (or HSA), a small molecule derived from naturally produced HS that is emerging as a new alternative to the centuries-old aging dilemma. ■

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