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Hyaluronic acid
A key molecule in skin aging

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Abbreviations: UV, ultraviolet; ROS, reactive oxygen species; MMP, matrix metalloproteinase; HA, hyaluronic acid; GAG, glycosaminoglycan; ECM, extracellular matrix; HAS, hyaluronic acid synthases; HYAL, hyaluronidases; CD44, cluster of differentiation 44; RHAMM, receptor for HA-mediated motility; TGF, transforming growth factor

Skin aging is a multifactorial process consisting of two distinct and independent mechanisms: intrinsic and extrinsic aging. Youthful skin retains its turgor, resilience and pliability, among others, due to its high content of water. Daily external injury, in addition to the normal process of aging, causes loss of moisture. The key molecule involved in skin moisture is hyaluronic acid (HA) that has unique capacity in retaining water. There are multiple sites for the control of HA synthesis, deposition, cell and protein association and degradation, reflecting the complexity of HA metabolism. The enzymes that synthesize or catabolize HA and HA receptors responsible for many of the functions of HA are all multigene families with distinct patterns of tissue expression. Understanding the metabolism of HA in the different layers of the skin and the interactions of HA with other skin components will facilitate the ability to modulate skin moisture in a rational manner.

Skin Aging

Human skin aging is a complex biological process, not yet fully understood. It is the result of two biologically independent processes. The first is intrinsic or innate aging, an unpreventable process, which affects the skin in the same pattern as it affects all internal organs. The second is extrinsic aging, which is the result of exposure to external factors, mainly ultraviolet (UV) irradiation, that is also referred to as photoaging.[1] Intrinsic skin aging is influenced by hormonal changes that occur with age,[2] such as the gradual decreased production of sex hormones from the mid-twenties and the diminution of estrogens and progesterone associated with menopause. It is well established that the deficiency in estrogens and androgens results in collagen degradation, dryness, loss of elasticity, epidermal atrophy and wrinkling of the skin.[3]

Even though intrinsic and extrinsic skin aging are distinctive processes, they share similarities in molecular mechanisms. For example, reactive oxygen species (ROS), arising from oxidative cell metabolism, play a major role in both processes.[4] ROS in extrinsic or intrinsic skin aging induce the transcription factor c-Jun via mitogen-activated protein kinases (MAPK), leading to overexpression of matrix metalloproteinase (MMP)-1, MMP-3 and MMP-9 and prevention of the expression of procollagen-1.[5] Therefore, elevated levels of degraded collagen and reduced collagen synthesis are pathologies occurring in intrinsically aged as well as photoaged skin.

Skin aging is also associated with loss of skin moisture. The key molecule involved in skin moisture is hyaluronan or hyaluronic acid (HA), a glycosaminoglycan (GAG) with a unique capacity to bind and retain water molecules.[6] HA belongs to the extracellular matrix (ECM) molecules. During the past decades the constituents of the skin have been well characterized. In the beginning, most of the studies focused on the cells that comprise the skin layers, such as the epidermis, the dermis and the underlying subcutis. Recently, it is appreciated that ECM molecules that lie between cells, in addition to providing a constructive framework, they exert major effects on cellular function. These ECM molecules, although they appear amorphous by light microscopy, they form a highly organized structure, comprising mainly of GAG, proteoglycans, growth factors and structural proteins such as collagens. Yet, the predominant component of the skin ECM is HA.

Recent reviews have described the involvement of HA with respect to its role in angiogenesis,[7] reactive oxygen species,[8] chondrocytes,[9] cancer,[10,11] lung injury,[12,13] immune regulation[14,15] and skin.[16] This review presents in brief recent knowledge in HA biology and function and focuses on its involvement in skin aging.

Hyaluronic Acid

Chemistry and physicochemical properties. HA is a non-sulphated GAG and is composed of repeating polymeric disaccharides of D-glucuronic acid and N-acetyl-D-glucosamine linked by a glucuronidic β (1→3) bond.[17,18] In aqueous solutions HA forms specific stable tertiary structures.[19] Despite the simplicity in its composition, without variations in its sugar composition or without branching points, HA has a variety of physicochemical properties. HA polymers occur in a vast number of configurations

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and shapes, depending on their size, salt concentration, pH, and associated cations.[20] Unlike other GAG, HA is not covalently attached to a protein core, but it may form aggregates with proteoglycans.[21] HA encompasses a large volume of water giving solutions high viscosity, even at low concentrations.[13]

Tissue and cell distribution of HA. HA is widely distributed, from prokaryotic,[22,23] to eukaryotic cells.[24] In humans, HA is most abundant in the skin,[25-29] accounting for 50% of the total body HA,[30] the vitreous of the eye,[31] the umbilical cord,[17] and synovial fluid,[32,33] but it is also present in all tissues and fluids of the body, such as skeletal tissues,[27] heart valves,[34] the lung,[35-39] the aorta,[40] the prostate,[41] tunica albuginea, corpora cavernosa and corpus spongiosum of the penis.[42] HA is produced primarily by mesenchymal cells but also by other cell types.[34-38,43]

Biological function of HA. Over the past two decades there was considerable evidence presented that unraveled the functional role of HA in molecular mechanisms and indicated the potential role of HA for the development of novel therapeutic strategies for many diseases.

Functions of HA include the following: hydration, lubrication of joints, a space filling capacity, and the framework through which cells migrate.[34] The synthesis of HA increases during tissue injury and wound healing[25,44,45] and HA regulates several aspects of tissue repair, including activation of inflammatory cells to enhance immune response[46-48] and the response to injury of fibroblasts[49,50] and epithelial cells.[51-55] HA also provides the framework for blood vessel formation[7,45] and fibroblast migration,[56,57] that may be involved in tumor progression.[58] The correlation of HA levels on the cell surface of cancer cells with the aggressiveness of tumors has also been reported.[59]

The size of HA appears to be of critical importance for its various functions described above. HA of high molecular size, usually in excess of 1,000 kDa, is present in intact tissues and is antiangiogenic and immunosuppressive, whereas smaller polymers of HA are distress signals and potent inducers of inflammation and angiogenesis.[38,46,60-63]

Biosynthesis of HA

HA is synthesized by specific enzymes called HA synthases (HAS). These are membrane bound enzymes that synthesize HA on the inner surface of the plasma membrane[64] and then HA is extruded through pore-like structures into the extracellular space.[24,65] There are three mammalian enzymes HAS -1, -2 and -3, which exhibit distinct enzymatic properties and synthesize HA chains of various length.[66-68]

Degradation of HA

HA has a dynamic turnover rate. HA has a half-life of 3 to 5 min in the blood, less than a day in the skin and 1 to 3 weeks in the cartilage.[69-71] HA is degraded into fragments of varying size by hyaluronidases (HYAL) by hydrolyzing the hexosaminidic β (1–4) linkages between N-acetyl-D-glucosamine and D-glucuronic acid residues in HA. In humans, six HYAL have been identified so far: HYAL-1, -2, -3, -4, PH-20 and HYALP1.[72] The family of HYAL enzymes received little attention until recently[73,74] because they are found at extremely low concentrations and they are difficult to purify, characterize and measure their activity, which is high but unstable.[16] New procedures have now enabled the isolation and characterization of HYAL.[75,76] HYAL-1 is the major HYAL in serum.[77] Mutations in the HYAL-1 gene are associated with HYAL deficiency and mucopolysaccharidosis type IX.[78] HYAL-2 has very low activity in comparison to plasma HYAL-1 and it hydrolyzes specifically HA of high molecular weight, yielding HA fragments of approximately 20 kDa, which are further degraded to small oligosaccharides by PH-20.[79] HYAL-3 is mainly expressed in bone marrow and testis,[74] but also in other organs, such as the human lung.[37,38] The role of HYAL-3 in the catabolism of HA is not clear and it is suggested that it may contribute to HA degradation by enhancing the activity of HYAL-1.[80]

HA can also be degraded non-enzymatically by a free-radical mechanism[81] in the presence of reducing agents such as ascorbic acid, thiols, ferrous, or cuprous ions, a process that requires the presence of molecular oxygen. Thus, agents that could delay the free-radical-catalyzed degradation of HA may be useful in maintaining the integrity of dermal HA and its moisturizing properties.[16]

Hyaluronic Acid Receptors

There is a variety of proteins that bind HA, called hyaladherins, which are widely distributed in the ECM, the cell surface, the cytoplasm and the nucleus.[15] Those that attach HA to the cell surface constitute HA receptors. The most prominent among these receptors is the transmembrane glycoprotein "cluster of differentiation 44" (CD44) that occurs in many isoforms, which are the products of a single gene with variable exon expression.[82-84] CD44 is found on virtually all cells, except red blood cells, and regulates cell adhesion, migration, lymphocyte activation and homing, and cancer metastasis.

The receptor for HA-mediated motility (RHAMM) is another major receptor for HA, and it is expressed in various isoforms.[85-87] RHAMM is a functional receptor in many cell types, including endothelial cells[88] and in smooth muscle cells from human pulmonary arteries[37] and airways.[38] The interactions of HA with RHAMM control cell growth and migration by a complex network of signal transduction events and interactions with the cytoskeleton.[89] Transforming growth factor (TGF)-β1, which is a potent stimulator of cell motility, elicits the synthesis and expression of RHAMM and HA, and thus initiates locomotion.[90]

Hyaluronic Acid in Skin

The use of biotinylated HA-binding peptide[91] revealed that not only cells of mesenchymal origin were capable of synthesizing HA and permitted the histolocalization of HA in the dermal compartment of skin and the epidermis.[26,92-94] This technique enabled the visualization of HA in the epidermis, mainly in the ECM of the upper spinous and granular layers, whereas in the basal layer HA is predominantly intracellular.[26]

The function of the skin as a barrier is partly attributed to the lamellar bodies, thought to be modified lysosomes containing hydrolytic enzymes. They fuse with the plasma membranes of mature keratinocytes and they have the ability to acidify via proton pumps and partially convert their polar lipids into neutral lipids. Diffusion of aqueous material through the epidermis is blocked by these lipids synthesized by keratinocytes in the stratum granulosum. This boundary effect corresponds to the level of HA staining. The HA-rich area inferior to this layer may obtain water from the moisture-rich dermis, and the water contained therein cannot penetrate beyond the lipid-rich stratum granulosum. The hydration of the skin critically depends on the HA-bound water in the dermis and in the vital area of the epidermis, while maintenance of hydration essentially depends on the stratum granulosum. Extensive loss of the stratum granulosum in patients with burns may cause serious clinical problems due to dehydration.[16]

As mentioned above, skin HA accounts for most of 50% of total body HA.[30] The HA content of the dermis is significantly higher than that of the epidermis, while papillary dermis has much greater levels of HA than reticular dermis.[92] The HA of the dermis is in continuity with the lymphatic and vascular systems. HA in the dermis regulates water balance, osmotic pressure and ion flow and functions as a sieve, excluding certain molecules, enhancing the extracellular domain of cell surfaces and stabilizes skin structures by electrostatic interactions.[16] Elevated levels of HA are synthesized during scar-free fetal tissue repair and the prolonged presence of HA assures such scar-free tissue repair.[95-97] Dermal fibroblasts provide the synthetic machinery for dermal HA and should be the target for pharmacologic attempts to enhance skin hydration. Unfortunately, exogenous HA is cleared from the dermis and is rapidly degraded.[70]

Hyaluronic acid synthases in the skin. In the skin, gene expression of HAS-1 and HAS-2 in the dermis and epidermis is differentially upregulated by TGF-β1, indicating that HAS isoforms are independently regulated and that the function of HA is different in the dermis and the epidermis.[16,98] The mRNA expression of HAS-2 and HAS-3 can be stimulated by keratinocyte growth factor, which activates keratinocyte migration and stimulates wound healing, leading to the accumulation of intermediate-sized HA in the culture medium and within keratinocytes. The migratory response of keratinocytes in wound healing is stimulated by increased synthesis of HA.[99] HAS-2 mRNA is also induced by IL-1β and TNFα in fibroblasts[100] and by epidermal growth factor in rat epidermal keratinocytes.[101]

Dysregulated expression of HA synthases has been reported during tissue injury.[102-104] HAS-2 and HAS-3 mRNA are significantly increased after skin injury in mice, leading to increased epidermal HA.[104] In juvenile hyaline fibromatosis, which is a rare autosomal recessive disease characterized by deposition of hyaline material and multiple skin lesions, there is a significant decreased expression of HAS-1 and HAS-3, accounting for the reduced synthesis of HA in skin lesions.[105] In dermal fibroblasts, where the HAS-2 is the predominant isoform, glucocorticoids inhibit HAS mRNA almost completely, suggesting a molecular basis of the decreased HA in atrophic skin as a result of local treatment with glucocorticoids.[16]

Hyaluronidases in the skin. In the skin, it has not been established which of the various HYAL controls the turnover of HA in the dermis and the epidermis. The elucidation of the biology of HYAL in the skin may offer novel pharmacological targets to confront age related turnover of HA in skin.

HA receptors in the skin. In the dermis and epidermis HA is co-localized with CD44. However, the exact CD44 variants in the different skin compartments have not yet been elucidated. CD44-HA interactions have been reported to mediate the binding of Langerhans cells to HA in the matrix surrounding keratinocytes by their CD44-rich surfaces, as they migrate through the epidermis.[106,107] RHAMM is also expressed in the human skin.[28,29] The TGF-β1 induced stimulation of fibroblast locomotion is mediated via RHAMM,[90] while overexpression of RHAMM can lead to the transformation of fibroblasts.[108]

Hyaluronic Acid and Skin Aging

The most dramatic histochemical change observed in senescent skin is the marked disappearance of epidermal HA, while HA is still present in the dermis.[92] The reasons for this change in HA homeostasis with aging is unknown. As mentioned above, the synthesis of epidermal HA is influenced by the underlying dermis and is under separate controls from the synthesis of dermal HA.[16,98] Progressive reduction of the size of the HA polymers in skin as a result of aging has also been reported.[109] Thus, the epidermis loses the principle molecule responsible for binding and retaining water molecules, resulting in loss of skin moisture. In the dermis, the major age-related change is the increasing avidity of HA with tissue structures with the concomitant loss of HA extractability. This parallels the progressive cross-linking of collagen and the steady loss of collagen extractability with age.[16] All of the above age related phenomena contribute to the apparent dehydration, atrophy and loss of elasticity that characterizes aged skin.

Premature aging of skin is the result of repeated and extended exposure to UV radiation.[110,111] Approximately 80% of facial skin aging is attributed to UV-exposure.[112] UV radiation damage causes initially a mild form of wound healing and is associated at first with an increase of dermal HA. As little as 5 min of UV exposure in nude mice caused enhanced deposition of HA, indicating that UV radiation induced skin damage is an extremely rapid event.[16] The initial redness of the skin following exposure to UV radiation may be due to a mild edematous reaction induced by the enhanced HA deposition and histamine release. Repeated and extensive exposures to UV ultimately simulate a typical wound healing response with deposition of scarlike type I collagen, rather than the usual types I and III collagen mixture that gives skin resilience and pliability.[16]

In the skin, photoaging results in abnormal GAG content and distribution compared with that found in scars, or in the wound healing response, with diminished HA and increased levels of chondroitin sulfate proteoglycans.[111] In dermal fibroblasts this reduction in HA synthesis was attributed to collagen fragments, which activate $\alpha_v\beta_3$-integrins and in turn inhibit Rho kinase signaling and nuclear translocation of phosphoERK, resulting in

reduced HAS-2 expression.[113] We have recently unraveled some of the biochemical changes that may distinguish photoaging and natural aging. Using photoexposed and photoprotected human skin tissue specimens, obtained from the same patient, we have shown a significant increase in the expression of HA of lower molecular mass in photoexposed skin, as compared with photoprotected skin. This increase of degraded HA was associated with a significant decrease in the expression of HAS-1 and an increased expression of HYAL-1, -2 and -3. Furthermore, the expression of HA receptors CD44 and RHAMM was significantly downregulated in photoexposed, as compared with photoprotected skin. These findings indicate that photoexposed skin, and therefore extrinsic skin aging, is characterized by distinct homeostasis of HA.[29] We have also assessed photoprotected skin tissue specimens from adults and juvenile patients and observed that intrinsic skin aging was associated with a significant reduction in the content of HA and downregulation of HAS-1, HAS -2, CD44 and RHAMM.[28] Similar results for photoprotected skin have also been reported for both genders for HA, HAS-2 and CD44.[114]

Conclusion

The available data suggest that HA homeostasis exhibits a distinct profile in intrinsic skin aging, which is totally different of that in extrinsic skin aging. Additional insight needs to be gained in understanding the metabolism of HA in skin layers and the interactions of HA with other skin components. Such information will facilitate the ability to modulate skin moisture in a rational manner and may contribute to the refinement of current drugs and the development of novel treatments for skin aging.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Berneburg M, Trelles M, Friguet B, Ogden S, Esrefoglu M, Kaya G, et al. How best to halt and/or revert UV-induced skin ageing: strategies, facts and fiction. Exp Dermatol 2008; 17:228-40; PMID:18261088
2. Makrantonaki E, Adjaye J, Herwig R, Brink TC, Groth D, Hultschig C, et al. Age-specific hormonal decline is accompanied by transcriptional changes in human sebocytes in vitro. Aging Cell 2006; 5:331-44; PMID:16805856; http://dx.doi.org/10.1111/j.1474-9726.2006.00223.x
3. Brincat MP. Hormone replacement therapy and the skin. Maturitas 2000; 35:107–117. 9 Makrantonaki E, Zouboulis CC. Androgens and aging of the skin. Curr Opin Endocrinol Diabetes Obes 2009; 16:240-5; PMID:19390323
4. Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S, et al. Mechanisms of photoaging and chronological skin aging. Arch Dermatol 2002; 138:1462-70; PMID:12437452; http://dx.doi.org/10.1001/archderm.138.11.1462
5. Chung JH, Kang S, Varani J, Lin J, Fisher GJ, Voorhees JJ. Decreased extracellular-signal-regulated kinase and increased stress-activated MAP kinase activities in aged human skin in vivo. J Invest Dermatol 2000; 115:177-82; PMID:10951233; http://dx.doi.org/10.1046/j.1523-1747.2000.00009.x
6. Baumann L. Skin ageing and its treatment. J Pathol 2007; 211:241-51; PMID:17200942; http://dx.doi.org/10.1002/path.2098
7. Slevin M, Krupinski J, Gaffney J, Matou S, West D, Delisser H, et al. Hyaluronan-mediated angiogenesis in vascular disease: uncovering RHAMM and CD44 receptor signaling pathways. Matrix Biol 2007; 26:58-68; PMID:17055233; http://dx.doi.org/10.1016/j.matbio.2006.08.261
8. Soltés L, Mendichi R, Kogan G, Schiller J, Stankovska M, Arnhold J. Degradative action of reactive oxygen species on hyaluronan. Biomacromolecules 2006; 7:659-68; PMID:16529395; http://dx.doi.org/10.1021/bm050867v
9. Knudson CB, Knudson W. Hyaluronan and CD44: modulators of chondrocyte metabolism. Clin Orthop Relat Res 2004; (Suppl):S152-62; PMID:15480059; http://dx.doi.org/10.1097/01.blo.0000143804.26638.82
10. Toole BP, Zoltan-Jones A, Misra S, Ghatak S. Hyaluronan: a critical component of epithelial-mesenchymal and epithelial-carcinoma transitions. Cells Tissues Organs 2005; 179:66-72; PMID:15942194; http://dx.doi.org/10.1159/000084510
11. Toole BP, Ghatak S, Misra S. Hyaluronan oligosaccharides as a potential anticancer therapeutic. Curr Pharm Biotechnol 2008; 9:249-52; PMID:18691085; http://dx.doi.org/10.2174/138920108785161569
12. Noble PW. Hyaluronan and its catabolic products in tissue injury and repair. Matrix Biol 2002; 21:25-9; PMID:11827789; http://dx.doi.org/10.1016/S0945-053X(01)00184-6
13. Turino GM, Cantor JO. Hyaluronan in respiratory injury and repair. Am J Respir Crit Care Med 2003; 167:1169-75; PMID:12714341; http://dx.doi.org/10.1164/rccm.200205-449PP
14. Jackson DG. Immunological functions of hyaluronan and its receptors in the lymphatics. Immunol Rev 2009; 230:216-31; PMID:19594639; http://dx.doi.org/10.1111/j.1600-065X.2009.00803.x
15. Jiang D, Liang J, Noble PW. Hyaluronan as an immune regulator in human diseases. Physiol Rev 2011; 91:221-64; PMID:21248167; http://dx.doi.org/10.1152/physrev.00052.2009
16. Stern R, Maibach HI. Hyaluronan in skin: aspects of aging and its pharmacologic modulation. Clin Dermatol 2008; 26:106-22; PMID:18472055; http://dx.doi.org/10.1016/j.clindermatol.2007.09.013
17. Weissmann B, Meyer K. The structure of hyalobiuronic acid and of hyaluronic acid from umbilical cord. J Am Chem Soc 1954; 76:1753-7; http://dx.doi.org/10.1021/ja01636a010
18. Weissmann B, Meyer K, Sampson P, Linker A. Isolation of oligosaccharides enzymatically produced from hyaluronic acid. J Biol Chem 1954; 208:417-29; PMID:13174551
19. Scott JE, Heatley F. Hyaluronan forms specific stable tertiary structures in aqueous solution: a 13C NMR study. Proc Natl Acad Sci U S A 1999; 96:4850-5; PMID:10220382; http://dx.doi.org/10.1073/pnas.96.9.4850
20. Laurent TC. Structure of hyaluronic acid. In: Balazs, EA, ed. Chemistry and Molecular Biology of the Intercellular Matrix, Academic Press: New York, 1970:p. 703
21. Bates EJ, Harper GS, Lowther DA, Preston BN. Effect of oxygen-derived reactive species on cartilage proteoglycan-hyaluronate aggregates. Biochem Int 1984; 8:629-37; PMID:6548142
22. Lowther DA, Rogers HJ. Biosynthesis of hyaluronate. Nature 1955; 175:435; PMID:14356201; http://dx.doi.org/10.1038/175435a0
23. MacLennan AP. The production of capsules, hyaluronic acid and hyaluronidase by 25 strains of group C streptococci. J Gen Microbiol 1956; 15:485-91; PMID:13385432; http://dx.doi.org/10.1099/00221287-15-3-485
24. Prehm P. Release of hyaluronate from eukaryotic cells. Biochem J 1990; 267:185-9; PMID:2158307
25. Juhlin L. Hyaluronan in skin. J Intern Med 1997; 242:61-6; PMID:9260568; http://dx.doi.org/10.1046/j.1365-2796.1997.00175.x
26. Tammi R, Ripellino JA, Margolis RU, Tammi M. Localization of epidermal hyaluronic acid using the hyaluronate binding region of cartilage proteoglycan as a specific probe. J Invest Dermatol 1988; 90:412-4; PMID:2450149; http://dx.doi.org/10.1111/1523-1747.ep12456530
27. Armstrong SE, Bell DR. Relationship between lymph and tissue hyaluronan in skin and skeletal muscle. Am J Physiol Heart Circ Physiol 2002; 283:H2485-94; PMID:12388305
28. Tzellos TG, Sinopidis X, Kyrgidis A, Vahtsevanos K, Triaridis S, Printza A, et al. Differential hyaluronan homeostasis and expression of proteoglycans in juvenile and adult human skin. J Dermatol Sci 2011; 61:69-72; PMID:21087840; http://dx.doi.org/10.1016/j.jdermsci.2010.10.010
29. Tzellos TG, Klagas I, Vahtsevanos K, Triaridis S, Printza A, Kyrgidis A, et al. Extrinsic ageing in the human skin is associated with alterations in the expression of hyaluronic acid and its metabolizing enzymes. Exp Dermatol 2009; 18:1028-35; PMID:19601984; http://dx.doi.org/10.1111/j.1600-0625.2009.00889.x
30. Reed RK, Lilja K, Laurent TC. Hyaluronan in the rat with special reference to the skin. Acta Physiol Scand 1988; 134:405-11; PMID:3227957; http://dx.doi.org/10.1111/j.1748-1716.1988.tb08508.x
31. Meyer K, Palmer JW. The Polysaccharide of the vitreous humor. J Biol Chem 1934; 107:629-34
32. Hamerman D, Schuster H. Hyaluronate in normal human synovial fluid. J Clin Invest 1958; 37:57-64; PMID:13491713; http://dx.doi.org/10.1172/JCI103585
33. Ragan C, Meyer K. The hyaluronic acid of synovial fluid in rheumatoid arthritis. J Clin Invest 1949; 28:56-9; http://dx.doi.org/10.1172/JCI102053
34. Toole BP. Hyaluronan: from extracellular glue to pericellular cue. Nat Rev Cancer 2004; 4:528-39; PMID:15229478; http://dx.doi.org/10.1038/nrc1391
35. Papakonstantinou E, Karakiulakis G, Roth M, Block LH. Platelet-derived growth factor stimulates the secretion of hyaluronic acid by proliferating human vascular smooth muscle cells. Proc Natl Acad Sci U S A 1995; 92:9881-5; PMID:7568237; http://dx.doi.org/10.1073/pnas.92.21.9881

36. Papakonstantinou E, Roth M, Tamm M, Eickelberg O, Perruchoud AP, Karakiulakis G. Hypoxia differentially enhances the effects of transforming growth factor-beta isoforms on the synthesis and secretion of glycosaminoglycans by human lung fibroblasts. J Pharmacol Exp Ther 2002; 301:830-7; PMID:12023510; http://dx.doi.org/10.1124/jpet.301.3.830
37. Papakonstantinou E, Kouri FM, Karakiulakis G, Klagas I, Eickelberg O. Increased hyaluronic acid content in idiopathic pulmonary arterial hypertension. Eur Respir J 2008; 32:1504-12; PMID:18768572; http://dx.doi.org/10.1183/09031936.00159507
38. Klagas I, Goulet S, Karakiulakis G, Zhong J, Baraket M, Black JL, et al. Decreased hyaluronan in airway smooth muscle cells from patients with asthma and COPD. Eur Respir J 2009; 34:616-28; PMID:19282346; http://dx.doi.org/10.1183/09031936.00070808
39. Papakonstantinou E, Karakiulakis G. The 'sweet' and 'bitter' involvement of glycosaminoglycans in lung diseases: pharmacotherapeutic relevance. Br J Pharmacol 2009; 157:1111-27; PMID:19508395; http://dx.doi.org/10.1111/j.1476-5381.2009.00279.x
40. Papakonstantinou E, Karakiulakis G, Eickelberg O, Perruchoud AP, Block LH, Roth M. A 340 kDa hyaluronic acid secreted by human vascular smooth muscle cells regulates their proliferation and migration. Glycobiology 1998; 8:821-30; PMID:9639543; http://dx.doi.org/10.1093/glycob/8.8.821
41. Goulas A, Hatzichristou DG, Karakiulakis G, Mirtsou-Fidani V, Kalinderis A, Papakonstantinou E. Benign hyperplasia of the human prostate is associated with tissue enrichment in chondroitin sulphate of wide size distribution. Prostate 2000; 44:104-10; PMID:10881019; http://dx.doi.org/10.1002/1097-0045(20000701)44:2<104::AID-PROS2>3.0.CO;2-6
42. Goulas A, Papakonstantinou E, Karakiulakis G, Mirtsou-Fidani V, Kalinderis A, Hatzichristou DG. Tissue structure-specific distribution of glycosaminoglycans in the human penis. Int J Biochem Cell Biol 2000; 32:975-82; PMID:11084377; http://dx.doi.org/10.1016/S1357-2725(00)00038-8
43. Lee JY, Spicer AP. Hyaluronan: a multifunctional, megaDalton, stealth molecule. Curr Opin Cell Biol 2000; 12:581-6; PMID:10978893; http://dx.doi.org/10.1016/S0955-0674(00)00135-6
44. Weigel PH, Fuller GM, LeBoeuf RD. A model for the role of hyaluronic acid and fibrin in the early events during the inflammatory response and wound healing. J Theor Biol 1986; 119:219-34; PMID:3736072; http://dx.doi.org/10.1016/S0022-5193(86)80076-5
45. Slevin M, Kumar S, Gaffney J. Angiogenic oligosaccharides of hyaluronan induce multiple signaling pathways affecting vascular endothelial cell mitogenic and wound healing responses. J Biol Chem 2002; 277:41046-59; PMID:12194965; http://dx.doi.org/10.1074/jbc.M109443200
46. McKee CM, Penno MB, Cowman M, Burdick MD, Strieter RM, Bao C, et al. Hyaluronan (HA) fragments induce chemokine gene expression in alveolar macrophages. The role of HA size and CD44. J Clin Invest 1996; 98:2403-13; PMID:8941660; http://dx.doi.org/10.1172/JCI119054
47. Horton MR, McKee CM, Bao C, Liao F, Farber JM, Hodge-DuFour J, et al. Hyaluronan fragments synergize with interferon-gamma to induce the C-X-C chemokines mig and interferon-inducible protein-10 in mouse macrophages. J Biol Chem 1998; 273:35088-94; PMID:9857043; http://dx.doi.org/10.1074/jbc.273.52.35088
48. Teriete P, Banerji S, Noble M, Blundell CD, Wright AJ, Pickford AR, et al. Structure of the regulatory hyaluronan binding domain in the inflammatory leukocyte homing receptor CD44. Mol Cell 2004; 13:483-96; PMID:14992719; http://dx.doi.org/10.1016/S1097-2765(04)00080-2
49. Itano N, Atsumi F, Sawai T, Yamada Y, Miyaishi O, Senga T, et al. Abnormal accumulation of hyaluronan matrix diminishes contact inhibition of cell growth and promotes cell migration. Proc Natl Acad Sci U S A 2002; 99:3609-14; PMID:11891291; http://dx.doi.org/10.1073/pnas.052026799
50. Bai KJ, Spicer AP, Mascarenhas MM, Yu L, Ochoa CD, Garg HG, et al. The role of hyaluronan synthase 3 in ventilator-induced lung injury. Am J Respir Crit Care Med 2005; 172:92-8; PMID:15790861; http://dx.doi.org/10.1164/rccm.200405-652OC
51. Beck-Schimmer B, Oertli B, Pasch T, Wüthrich RP. Hyaluronan induces monocyte chemoattractant protein-1 expression in renal tubular epithelial cells. J Am Soc Nephrol 1998; 9:2283-90; PMID:9848782
52. Zoltan-Jones A, Huang L, Ghatak S, Toole BP. Elevated hyaluronan production induces mesenchymal and transformed properties in epithelial cells. J Biol Chem 2003; 278:45801-10; PMID:12954618; http://dx.doi.org/10.1074/jbc.M308168200
53. Jameson JM, Cauvi G, Sharp LL, Witherden DA, Havran WL. Gammadelta T cell-induced hyaluronan production by epithelial cells regulates inflammation. J Exp Med 2005; 201:1269-79; PMID:15837812; http://dx.doi.org/10.1084/jem.20042057
54. Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, et al. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat Med 2005; 11:1173-9; PMID:16244651; http://dx.doi.org/10.1038/nm1315
55. Jiang D, Liang J, Li Y, Noble PW. The role of Toll-like receptors in non-infectious lung injury. Cell Res 2006; 16:693-701; PMID:16894359; http://dx.doi.org/10.1038/sj.cr.7310085
56. Li L, Heldin CH, Heldin P. Inhibition of platelet-derived growth factor-BB-induced receptor activation and fibroblast migration by hyaluronan activation of CD44. J Biol Chem 2006; 281:26512-9; PMID:16809345; http://dx.doi.org/10.1074/jbc.M605607200
57. Turley EA. The role of a cell-associated hyaluronan-binding protein in fibroblast behaviour. Ciba Found Symp 1989; 143:121-33, discussion 133-7, 281-5; PMID:2478343
58. Knudson W. Tumor-associated hyaluronan. Providing an extracellular matrix that facilitates invasion. Am J Pathol 1996; 148:1721-6; PMID:8669457
59. Zhang L, Underhill CB, Chen L. Hyaluronan on the surface of tumor cells is correlated with metastatic behavior. Cancer Res 1995; 55:428-33; PMID:7529138
60. West DC, Hampson IN, Arnold F, Kumar S. Angiogenesis induced by degradation products of hyaluronic acid. Science 1985; 228:1324-6; PMID:2408340; http://dx.doi.org/10.1126/science.2408340
61. McKee CM, Lowenstein CJ, Horton MR, Wu J, Bao C, Chin BY, et al. Hyaluronan fragments induce nitric-oxide synthase in murine macrophages through a nuclear factor kappaB-dependent mechanism. J Biol Chem 1997; 272:8013-8; PMID:9065473; http://dx.doi.org/10.1074/jbc.272.12.8013
62. Termeer CC, Hennies J, Voith U, Ahrens T, Weiss JM, Prehm P, et al. Oligosaccharides of hyaluronan are potent activators of dendritic cells. J Immunol 2000; 165:1863-70; PMID:10925265
63. Papakonstantinou E, Klagas I, Karakiulakis G, Hostettler K, S'ng CT, Kotoula V, et al. Steroids and β2 Agonists Regulate Hyaluronan Metabolism in Asthma Airway Smooth Muscle Cells. Am J Respir Cell Mol Biol 2012; In press; PMID:22865625; http://dx.doi.org/10.1165/rcmb.2012-0101OC
64. Prehm P. Hyaluronate is synthesized at plasma membranes. Biochem J 1984; 220:597-600; PMID:6743290
65. Watanabe K, Yamaguchi Y. Molecular identification of a putative human hyaluronan synthase. J Biol Chem 1996; 271:22945-8; PMID:8798477; http://dx.doi.org/10.1074/jbc.271.38.22945
66. Weigel PH, Hascall VC, Tammi M. Hyaluronan synthases. J Biol Chem 1997; 272:13997-4000; PMID:9206724; http://dx.doi.org/10.1074/jbc.272.22.13997
67. Itano N, Sawai T, Yoshida M, Lenas P, Yamada Y, Imagawa M, et al. Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties. J Biol Chem 1999; 274:25085-92; PMID:10455188; http://dx.doi.org/10.1074/jbc.274.35.25085
68. Itano N, Kimata K. Mammalian hyaluronan synthases. IUBMB Life 2002; 54:195-9; PMID:12512858; http://dx.doi.org/10.1080/15216540214929
69. Fraser JR, Laurent TC, Pertoft H, Baxter E. Plasma clearance, tissue distribution and metabolism of hyaluronic acid injected intravenously in the rabbit. Biochem J 1981; 200:415-24; PMID:7340841
70. Reed RK, Laurent UB, Fraser JR, Laurent TC. Removal rate of [3H]hyaluronan injected subcutaneously in rabbits. Am J Physiol 1990; 259:H532-5; PMID:2386226
71. Laurent UB, Dahl LB, Reed RK. Catabolism of hyaluronan in rabbit skin takes place locally, in lymph nodes and liver. Exp Physiol 1991; 76:695-703; PMID:1742011
72. Stern R, Jedrzejas MJ. Hyaluronidases: their genomics, structures, and mechanisms of action. Chem Rev 2006; 106:818-39; PMID:16522010; http://dx.doi.org/10.1021/cr050247k
73. Kreil G. Hyaluronidases--a group of neglected enzymes. Protein Sci 1995; 4:1666-9; PMID:8528065; http://dx.doi.org/10.1002/pro.5560040902
74. Csoka AB, Frost GI, Stern R. The six hyaluronidase-like genes in the human and mouse genomes. Matrix Biol 2001; 20:499-508; PMID:11731267; http://dx.doi.org/10.1016/S0945-053X(01)00172-X
75. Frost GI, Stern R. A microtiter-based assay for hyaluronidase activity not requiring specialized reagents. Anal Biochem 1997; 251:263-9; PMID:9299025; http://dx.doi.org/10.1006/abio.1997.2262
76. Guntenhöner MW, Pogrel MA, Stern R. A substrate-gel assay for hyaluronidase activity. Matrix 1992; 12:388-96; PMID:1484506; http://dx.doi.org/10.1016/S0934-8832(11)80035-1
77. Chichibu K, Matsuura T, Shichijo S, Yokoyama MM. Assay of serum hyaluronic acid in clinical application. Clin Chim Acta 1989; 181:317-23; PMID:2474393; http://dx.doi.org/10.1016/0009-8981(89)90237-4
78. Natowicz MR, Short MP, Wang Y, Dickersin GR, Gebhardt MC, Rosenthal DI, et al. Clinical and biochemical manifestations of hyaluronidase deficiency. N Engl J Med 1996; 335:1029-33; PMID:8793927; http://dx.doi.org/10.1056/NEJM199610033351405
79. Lepperdinger G, Strobl B, Kreil G. HYAL2, a human gene expressed in many cells, encodes a lysosomal hyaluronidase with a novel type of specificity. J Biol Chem 1998; 273:22466-70; PMID:9712871; http://dx.doi.org/10.1074/jbc.273.35.22466
80. Hemming R, Martin DC, Slominski E, Nagy JI, Halayko AJ, Pind S, et al. Mouse Hyal3 encodes a 45- to 56-kDa glycoprotein whose overexpression increases hyaluronidase 1 activity in cultured cells. Glycobiology 2008; 18:280-9; PMID:18234732; http://dx.doi.org/10.1093/glycob/cwn006
81. Lapcík L Jr., Chabrecek P, Stasko A. Photodegradation of hyaluronic acid: EPR and size exclusion chromatography study. Biopolymers 1991; 31:1429-35; PMID:1667853; http://dx.doi.org/10.1002/bip.360311209
82. Laurent TC. The chemistry, biology, and medical applications of hyaluronan and its derivatives. London: Portland Press; 1998:621
83. Toole BP. Hyaluronan and its binding proteins, the hyaladherins. Curr Opin Cell Biol 1990; 2:839-44; PMID:1707285; http://dx.doi.org/10.1016/0955-0674(90)90081-O
84. Knudson CB, Knudson W. Hyaluronan-binding proteins in development, tissue homeostasis, and disease. FASEB J 1993; 7:1233-41; PMID:7691670

85. Turley EA. Hyaluronan and cell locomotion. Cancer Metastasis Rev 1992; 11:21-30; PMID:1380898; http://dx.doi.org/10.1007/BF00047600
86. Hardwick C, Hoare K, Owens R, Hohn HP, Hook M, Moore D, et al. Molecular cloning of a novel hyaluronan receptor that mediates tumor cell motility. J Cell Biol 1992; 117:1343-50; PMID:1376732; http://dx.doi.org/10.1083/jcb.117.6.1343
87. Yang B, Zhang L, Turley EA. Identification of two hyaluronan-binding domains in the hyaluronan receptor RHAMM. J Biol Chem 1993; 268:8617-23; PMID:7682552
88. Lokeshwar VB, Selzer MG. Differences in hyaluronic acid-mediated functions and signaling in arterial, microvessel, and vein-derived human endothelial cells. J Biol Chem 2000; 275:27641-9; PMID:10882722
89. Mohapatra S, Yang X, Wright JA, Turley EA, Greenberg AH. Soluble hyaluronan receptor RHAMM induces mitotic arrest by suppressing Cdc2 and cyclin B1 expression. J Exp Med 1996; 183:1663-8; PMID:8666924; http://dx.doi.org/10.1084/jem.183.4.1663
90. Samuel SK, Hurta RA, Spearman MA, Wright JA, Turley EA, Greenberg AH. TGF-beta 1 stimulation of cell locomotion utilizes the hyaluronan receptor RHAMM and hyaluronan. J Cell Biol 1993; 123:749-58; PMID:7693717; http://dx.doi.org/10.1083/jcb.123.3.749
91. Ripellino JA, Bailo M, Margolis RU, Margolis RK. Light and electron microscopic studies on the localization of hyaluronic acid in developing rat cerebellum. J Cell Biol 1988; 106:845-55; PMID:2450100; http://dx.doi.org/10.1083/jcb.106.3.845
92. Meyer LJ, Stern R. Age-dependent changes of hyaluronan in human skin. J Invest Dermatol 1994; 102:385-9; PMID:8120424; http://dx.doi.org/10.1111/1523-1747.ep12371800
93. Wang C, Tammi M, Tammi R. Distribution of hyaluronan and its CD44 receptor in the epithelia of human skin appendages. Histochemistry 1992; 98:105-12; PMID:1429018; http://dx.doi.org/10.1007/BF00717001
94. Bertheim U, Hellström S. The distribution of hyaluronan in human skin and mature, hypertrophic and keloid scars. Br J Plast Surg 1994; 47:483-9; PMID:7524987; http://dx.doi.org/10.1016/0007-1226(94)90031-0
95. DePalma RL, Krummel TM, Durham LA 3rd, Michna BA, Thomas BL, Nelson JM, et al. Characterization and quantitation of wound matrix in the fetal rabbit. Matrix 1989; 9:224-31; PMID:2779482; http://dx.doi.org/10.1016/S0934-8832(89)80054-X
96. Mast BA, Flood LC, Haynes JH, DePalma RL, Cohen IK, Diegelmann RF, et al. Hyaluronic acid is a major component of the matrix of fetal rabbit skin and wounds: implications for healing by regeneration. Matrix 1991; 11:63-8; PMID:2027330; http://dx.doi.org/10.1016/S0934-8832(11)80228-3
97. Longaker MT, Chiu ES, Adzick NS, Stern M, Harrison MR, Stern R. Studies in fetal wound healing. V. A prolonged presence of hyaluronic acid characterizes fetal wound fluid. Ann Surg 1991; 213:292-6; PMID:2009010; http://dx.doi.org/10.1097/00000658-199104000-00003
98. Stuhlmeier KM, Pollaschek C. Differential effect of transforming growth factor beta (TGF-beta) on the genes encoding hyaluronan synthases and utilization of the p38 MAPK pathway in TGF-beta-induced hyaluronan synthase 1 activation. J Biol Chem 2004; 279:8753-60; PMID:14676202; http://dx.doi.org/10.1074/jbc.M303945200
99. Karvinen S, Pasonen-Seppänen S, Hyttinen JM, Pienimäki JP, Törrönen K, Jokela TA, et al. Keratinocyte growth factor stimulates migration and hyaluronan synthesis in the epidermis by activation of keratinocyte hyaluronan synthases 2 and 3. J Biol Chem 2003; 278:49495-504; PMID:14506240; http://dx.doi.org/10.1074/jbc.M310445200
100. Wilkinson TS, Potter-Perigo S, Tsoi C, Altman LC, Wight TN. Pro- and anti-inflammatory factors cooperate to control hyaluronan synthesis in lung fibroblasts. Am J Respir Cell Mol Biol 2004; 31:92-9; PMID:14764429; http://dx.doi.org/10.1165/rcmb.2003-0380OC
101. Pienimaki JP, Rilla K, Fulop C, Sironen RK, Karvinen S, Pasonen S, et al. Epidermal growth factor activates hyaluronan synthase 2 in epidermal keratinocytes and increases pericellular and intracellular hyaluronan. J Biol Chem 2001; 276:20428-35; PMID:11262389; http://dx.doi.org/10.1074/jbc.M007601200
102. Yung S, Thomas GJ, Davies M. Induction of hyaluronan metabolism after mechanical injury of human peritoneal mesothelial cells in vitro. Kidney Int 2000; 58:1953-62; PMID:11044215; http://dx.doi.org/10.1111/j.1523-1755.2000.00367.x
103. Li Y, Rahmanian M, Widström C, Lepperdinger G, Frost GI, Heldin P. Irradiation-induced expression of hyaluronan (HA) synthase 2 and hyaluronidase 2 genes in rat lung tissue accompanies active turnover of HA and induction of types I and III collagen gene expression. Am J Respir Cell Mol Biol 2000; 23:411-8; PMID:10970834
104. Tammi R, Pasonen-Seppänen S, Kolehmainen E, Tammi M. Hyaluronan synthase induction and hyaluronan accumulation in mouse epidermis following skin injury. J Invest Dermatol 2005; 124:898-905; PMID:15854028; http://dx.doi.org/10.1111/j.0022-202X.2005.23697.x
105. Tzellos TG, Dionyssopoulos A, Klagas I, Karakiulakis G, Lazaridis L, Papakonstantinou E. Differential glycosaminoglycan expression and hyaluronan homeostasis in juvenile hyaline fibromatosis. J Am Acad Dermatol 2009; 61:629-38; PMID:19559501; http://dx.doi.org/10.1016/j.jaad.2009.03.042
106. Weiss JM, Sleeman J, Renkl AC, Dittmar H, Termeer CC, Taxis S, et al. An essential role for CD44 variant isoforms in epidermal Langerhans cell and blood dendritic cell function. J Cell Biol 1997; 137:1137-47; PMID:9166413; http://dx.doi.org/10.1083/jcb.137.5.1137
107. Weiss JM, Renkl AC, Sleeman J, Dittmar H, Termeer CC, Taxis S, et al. CD44 variant isoforms are essential for the function of epidermal Langerhans cells and dendritic cells. Cell Adhes Commun 1998; 6:157-60; PMID:9823467; http://dx.doi.org/10.3109/15419069809004472
108. Hall CL, Yang B, Yang X, Zhang S, Turley M, Samuel S, et al. Overexpression of the hyaluronan receptor RHAMM is transforming and is also required for H-ras transformation. Cell 1995; 82:19-26; PMID:7541721; http://dx.doi.org/10.1016/0092-8674(95)90048-9
109. Longas MO, Russell CS, He XY. Evidence for structural changes in dermatan sulfate and hyaluronic acid with aging. Carbohydr Res 1987; 159:127-36; PMID:3829041; http://dx.doi.org/10.1016/S0008-6215(00)90010-7
110. Gilchrest BA. A review of skin ageing and its medical therapy. Br J Dermatol 1996; 135:867-75; PMID:8977705; http://dx.doi.org/10.1046/j.1365-2133.1996.d01-1088.x
111. Bernstein EF, Underhill CB, Hahn PJ, Brown DB, Uitto J. Chronic sun exposure alters both the content and distribution of dermal glycosaminoglycans. Br J Dermatol 1996; 135:255-62; PMID:8881669; http://dx.doi.org/10.1111/j.1365-2133.1996.tb01156.x
112. Uitto J. Understanding premature skin aging. N Engl J Med 1997; 337:1463-5; PMID:9358147; http://dx.doi.org/10.1056/NEJM199711133372011
113. Röck K, Grandoch M, Majora M, Krutmann J, Fischer JW. Collagen fragments inhibit hyaluronan synthesis in skin fibroblasts in response to ultraviolet B (UVB): new insights into mechanisms of matrix remodeling. J Biol Chem 2011; 286:18268-76; PMID:21454612; http://dx.doi.org/10.1074/jbc.M110.201665
114. Oh JH, Kim YK, Jung JY, Shin JE, Chung JH. Changes in glycosaminoglycans and related proteoglycans in intrinsically aged human skin in vivo. Exp Dermatol 2011; 20:454-6; PMID:21426414; http://dx.doi.org/10.1111/j.1600-0625.2011.01258.x