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Review:

Roles of hyaluronan in cardiovascular and nervous system disorders*

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Abstract: Hyaluronan is a widely occurring extracellular matrix molecule, which is not only a supporting structural component, but also an active regulator of cellular functions. The chemophysical and biological properties of hyaluronan are greatly affected by its molecular size and several hyaluronan-binding proteins, making hyaluronan a fascinating molecule with great functional diversity. This review summarizes our current understanding of the roles of hyaluronan in cardiovascular and nervous system disorders, such as atherosclerosis, myocardial infarction, and stroke, with the aim to provide a foundation for future research and clinical trials.

Key words: Hyaluronic acid; Extracellular matrix; Cardiovascular vessel; Heart; Brain
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1 Introduction

The extracellular space (ECS), consisting of the interstitial fluid and the extracellular matrix (ECM), is important for material exchange and signal communication between cells. It often makes a critical contribution to the regulation of cell function. In brain, the width of ECS is typically 30–40 nm, a width that differs between the awake, sleeping, and anesthetized states. It can be as small as 10 nm wide in ischemic regions (Thorne and Nicholson, 2006; Xie et al., 2013). Though scientists have been interested in the brain ECS for over 50 years, it is only in recent years, with the identification of ECM molecules, that the physiological and pathological roles of brain ECS/ECM have begun to be unveiled.

The brain ECM consists of hyaluronan, proteoglycans, and multiple connecting proteins. Hyaluronan, or hyaluronic acid, is a common ECM component throughout connective, epithelial, and neural tissues. Though once considered to be a simple, nonspecific "goo" component, it is now well recognized that hyaluronan has important effects on cell proliferation and migration. It participates deeply in multiple biological processes including inflammatory response, angiogenesis, wound healing, and tumor malignancy (Fraser et al., 1997). Hyaluronan products have also been used clinically in ophthalmologic surgery, orthopedic therapy, and stem-cell transplantation (Ballios et al., 2015; Moshayedi et al., 2016). In this review, we focus on cardiovascular and nervous system disorders, summarizing recent advances in the understanding of ECM function.

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2 Hyaluronan and its molecular interactions

2.1 Biosynthesis of hyaluronan

Hyaluronan is a glycosaminoglycan consisting of linearly repeated (β,1–4)-D-glucuronic acid-(β,1–4)-*N*-acetyl-D-glucosamine disaccharide units (Fig. 1). Three kinds of hyaluronan synthases (HAS1, 2, and 3) have been identified in vertebrates (Itano and Kimata, 2002). The HASs are transmembrane proteins that assemble hyaluronan chains on the cytoplasmic side and simultaneously transport it out of the cell. The biosynthesis of hyaluronan is very high during development and growth stages, but declines in most tissues in adults. However, most tissues retain the potential for synthesizing hyaluronan, and often producing hyaluronan promptly and extensively in response to physiological stimuli. Many kinds of cells produce hyaluronan, but its major source is mesenchymal cells: astrocytes in the central nervous system and fibroblasts in heart (Asher and Bignami, 1991; Bignami and Asher, 1992).

2.2 Metabolism of hyaluronan

The turnover of hyaluronan is quite rapid relative to the turnover of other ECM components such as collagen. About one-third of the hyaluronan in human skin is replaced daily. Huge hyaluronan molecules, often with molecular masses in the order of millions of Daltons, are first partially degraded and released into the interstitial fluid. These fragments are further degraded in lymphatic vessels before entering the blood. The circulating fragments in blood are finally endocytosed by hepatocytes to complete degradation. The half-life of hyaluronan in blood circulation is very short, measured in minutes (Fraser et al., 1997).

Six functional hyaluronidase genes have been identified in humans: *HYAL1*, *HYAL2*, *HYAL3*, *HYAL4*, *SPAM1/PH-20*, and *CEMIP/KIAA1199* (Csoka et al., 2001; Stern and Jedrzejas, 2006). There is one additional pseudogene, *HYAL6P/HYALP1*, produces no functional enzyme. HYAL1 and HYAL2 are the major hyaluronidases in most tissues. HYAL2 cleaves high-molecular-weight (HMW) hyaluronan at the cell surface. The resulting fragments are then internalized into endolysosomes, where they are further hydrolyzed by the lysosomal enzyme HYAL1. Under certain conditions, reactive oxygen species also contribute to the breakdown of ECM hyaluronan (Saari et al., 1993).

2.3 Hyaluronan-binding proteins

The molecular structure of hyaluronan is relatively simple, but by contrast, its biological roles are surprisingly versatile. The reason for this is the broad range of hyaluronan molecular masses (from thousands to millions of Daltons) and the many hyaluronan-binding proteins (HABPs). Known hyaluronan receptors include CD44, receptor of hyaluronan-mediated motility (RHAMM), lymphatic vessel endothelial hyaluronan receptor (LYVE-1), hyaluronan receptor for endocytosis (HARE), and layilin (Day and Prestwich, 2002; Jiang et al., 2011). CD44, RHAMM, and layilin are involved in hyaluronan-related signal transduction, cell activation, and locomotion. LYVE-1 and HARE are involved in the metabolism of hyaluronan. There are many more HABPs occurring throughout the body, with some being constitutive components of hyaluronan-rich ECM (aggrecan and link protein in joint cartilage and brevican in brain), and some occurring only when necessary (tumor necrosis factor-stimulated gene 6 (TSG-6) protein and serum-derived hyaluronan-associated protein (SHAP)) during inflammatory responses (Day and Prestwich, 2002; Zhuo et al., 2004). It is noteworthy that most HABPs bind to hyaluronan via non-covalent interactions, while SHAP binds via a covalent ester bond.

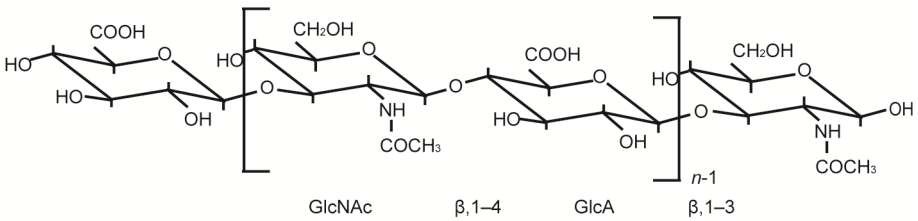

Fig. 1 Structure of hyaluronan

3 Physiological and pathological functions of hyaluronan

3.1 Construction of ECM

Hyaluronan is the most powerful water-binding molecule in our body; it can absorb up to 1000 times its mass in water (Day and Sheehan, 2001). This property makes it an extremely important space-filling and water-storing molecule in the ECM of tissues including joint cartilage, skin, and brain. In the brain, many neurons are coated by a perineuronal net that is a macroaggregate of hyaluronan, lecticans, tenascin-R, and other connecting proteins (Kwok et al., 2011) (Fig. 2).

3.2 Regulation of cell migration

Hyaluronan also participates in the construction of transient and dynamic ECM, where it participates in changing cell shape and accelerating cell migration. For example, before the smooth-muscle cells of blood vessels begin to migrate they produce more hyaluronan at their heads and tails, so that the steric exclusion effect of hyaluronan helps loosen the adhesion between the heads/tails and the substratum, thus facilitating cell migration (Zimmerman et al., 2002; Zoltan-Jones et al., 2003). This effect is also observed in metastasizing cancer cells and in skin epithelial cells migrating into an injured regions (Tammi et al., 2002; Schmaus et al., 2014). Hyaluronan mediates these effects, at least partially, by regulating the expression of cytoskeletal proteins, including vimentin and cytokeratin (Zoltan-Jones et al., 2003).

Low-molecular-weight (LMW) hyaluronan induces angiogenesis (West et al., 1985). Its significant molecular interactions include the recognition of LMW hyaluronan by CD44/RHAMM on the surface of endothelial cells, the activation of Src kinase and the MAPK(ERK-1/2) pathway, and the consequent upregulation of c-Myc protein (Savani et al., 2001; Yang et al., 2008). By contrast, HMW hyaluronan usually has an inhibitory effect on angiogenesis. This dependency of biological activity on molecular size is a unique characteristic of hyaluronan (McKee et al., 1996; Termeer et al., 2000; Rayahin et al., 2015).

3.3 Activation of inflammatory cells and immunoregulation

When tissues are injured, the ECM hyaluronan is often degraded, releasing LMW hyaluronan, a proinflammatory factor (Chajara et al., 2000). LMW hyaluronan activates multiple inflammatory and immune cells; for example, 50-kDa hyaluronan activates macrophages, renal tubule epithelial cells, and human bladder cancer T-24 cells, while LMW hyaluronan of 620 kDa stimulates dendritic cells to express inflammatory response-related genes including *IL-12*, *TGF-β1*, and *NF-κB* (Noble et al., 1993; Zimmerman et al., 2002; Khan et al., 2004). By contrast, HMW hyaluronan typically has anti-inflammatory effects (McKee et al., 1996; Rayahin et al., 2015).

Inflammatory responses stimulate the production of hyaluronan at lesioned sites and their neighboring tissues, which then contribute to the recruitment of hyaluronan receptor-bearing inflammatory

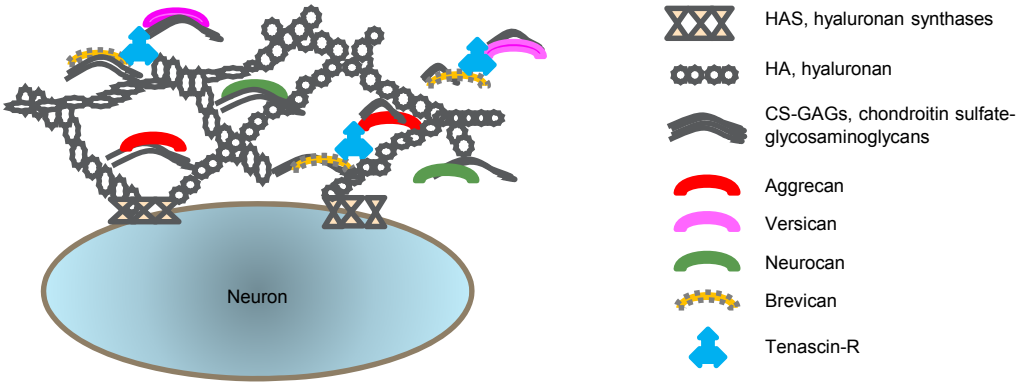

Fig. 2 Schematic of the perineuronal net

and immune cells. In CD44-deficient mice, the extravasation of leukocytes into extravascular inflammatory tissues is significantly compromised (Khan et al., 2004). Consistent with this observation, the blockage of CD44 by antibody binding prevents the development of edema that accompanies delayed-type hypersensitivity responses.

4 Hyaluronan in cardiovascular disorders

4.1 Embryonic cardiovascular development

Hyaluronan is indispensable for the characteristic transformation of cardiac endothelial cells into mesenchyme cells, which is an essential developmental event of cardiovascular formation. In Has2-deficient mice, the ECMs around embryonic cells become denser, and the migration of embryonic cells is blocked, resulting in severe cardiac and vascular abnormalities and finally death at midgestation (E9.5–10) (Camenisch et al., 2000). This defect can be reversed by gene rescue, hyaluronan supplementation, or expression of constitutively-active Ras mutants. On the contrary, Hyal2 deficiency causes an accumulation of hyaluronan and interstitial cells, resulting in congenital heart defects and heart failure (Chowdhury et al., 2017).

4.2 Hyaluronan in atherosclerosis

Atherosclerosis is a disorder in which plaque, made up of fat, cholesterol, calcium, and other substances, builds up inside arteries, eventually hardening and narrowing them, producing pathologies including ischemic heart attack, stroke, and death (Jonasson et al., 1986; Stary et al., 1995). Globally, ischemic heart attack and stroke are the two leading causes of total deaths and years of life lost in 2016 (GBD 2016 Causes of Death Collaborators, 2017). The progression of atherosclerosis has been separated into four stages: pathologic intimal thickening (PIT) without macrophage infiltration, intimal thickening with macrophage infiltration, early atheromatous plaques, and late fibroatheromas (Otsuka et al., 2015).

Hyaluronan is present in all layers of the aorta. With the progression of atherosclerosis, the percentage of area positive for hyaluronan staining in the lipid pool/necrotic core decreases to 33.4%, 54.4%, 28.1%, and 3.5% at the four stages described above, respectively (Otsuka et al., 2015). The hyaluronan binding proteoglycans, versican and biglycan, also decline in a similar pattern. Further investigation has shown that hyaluronan plays important roles in many critical events of plaque formation including lipid capture, immune activation, smooth-muscle cell proliferation, and macrophage recruitment, suggesting that hyaluronan is a key molecule in the pathogenesis of atherosclerosis (Viola et al., 2016).

Feeding apolipoprotein E (ApoE)-deficient mice with a high-fat diet induces atherosclerosis. Intravenous injection of them with hyaluronan nanoparticles (HA-NPs) decreased the amount of plaque macrophages by 30%. This is important because plaque macrophages exhibit 6- and 40-times higher uptake of HA-NPs than do splenic and bone marrow-resident macrophages, respectively (Beldman et al., 2017). HA-NPs have anti-inflammatory effects in atherosclerosis (Beldman et al., 2017). These data suggest that the development of atherosclerosis is accompanied by a loss of hyaluronan and HABPs, suggesting a possible treatment by administering HA-NPs.

It has also been reported that hyperglycemia upregulates hyaluronan production, causing changes in vascular smooth muscle cell subtype and cell proliferation that produce higher arterial hardness and stress. Such microenvironments are suitable for lipid deposition and leukocyte infiltration, leading to atherosclerosis (Lorentzen et al., 2016).

4.3 Myocardial infarction recovery

Infarct healing involves an initial inflammatory phase, in which leukocytes infiltrate to clear dead cells and matrix debris from the infarct, and a subsequent proliferative phase, in which myofibroblasts accumulate and deposit ECM proteins to form a collagen-based scar. In infarcted myocardium, CD44 expression was dramatically induced in infiltrating leukocytes, wound myofibroblasts, and vascular endothelial cells (Huebener et al., 2008). In CD44-deficient mice, the inflammatory phase was enhanced, as infiltration of neutrophils and macrophages increased by approximately 70%, while the proliferative phase was suppressed, with reduced fibroblast infiltration, collagen deposition, and proliferative activity. The resulting defective matrix network reduces the tensile strength of the ventricle and facilitates hypertrophy. The defects in healing from myocardial infarction in

the absence of CD44 are associated with an enhanced dilative remodeling of the infarcted ventricle, without affecting the size of the infarct (Huebener et al., 2008). These findings imply a way to improve the mechanical stress sensitivity of damaged cardiac myocytes via regulating the interaction of hyaluronan receptors with integrin (Chopra et al., 2012).

5 Hyaluronan in brain disorders

5.1 Hyaluronan as a biomarker in cerebrospinal fluid

Cerebrospinal fluid (CSF) is rich in hyaluronan, which originates mainly from the superficial layer of cerebral cortex, but not the choroid plexus and meninges (Laurent et al., 1996). Levels of CSF hyaluronan are affected by age, location, and pathology. Hyaluronan levels in lumbar CSF of healthy children and adults are (50±41) μg/L and (166±77) μg/L, respectively. Ventricular CSF of adults contains significantly less hyaluronan ((53±73) μg/L) than does lumbar CSF. Levels of CSF hyaluronan increase in patients with traumatic brain injury, cerebral infarction, subarachnoid hemorrhage, hydrocephalus, encephalitis, and meningitis, but not in those with multiple sclerosis (Laurent et al., 1996). The presence of intracranial primary tumors or metastases also dramatically alters levels of CSF hyaluronan, ranging from 13 to 2650 μg/L, and the size of hyaluronan averages 2.4×10^6 Da in brain tumor cyst fluid vs. 3×10^5 Da in healthy CSF (Laurent et al., 1996). Therefore, levels of CSF hyaluronan are a potential biomarker for monitoring disease progression and treatment efficacy.

5.2 Brain and spinal cord injury

Brain trauma significantly elevates levels of CSF hyaluronan (Laurent et al., 1996), also upregulating the expression of hyaluronan receptor CD44 and binding proteins brevican and versican (Xing et al., 2014). Upon brain puncture injury, CD44 appears around the lesion within two days and persists for two months, where it promotes the recruitment of T cells and the migration of astrocytes (Stylli et al., 2000). Hyaluronan also accumulates at the lesion, activating microglia and macrophages. All of these components participate in the regulation of immune responses after brain trauma. In addition, the combination of small hyaluronan fragment and Toll-like receptor 2 (TLR-2) can activate apoptosis (Tang et al., 2007). Exogenous hyaluronan tetrasaccharide (HA4) has been reported to significantly promote functional recovery after spinal cord injury (Wakao et al., 2011).

5.3 Stroke

Cerebral infarction and subarachnoid hemorrhage are associated with an increased hyaluronan in CSF (Laurent et al., 1996). Postmortem examinations of brains also showed an increase of total hyaluronan, particularly oligomeric hyaluronan, consisting of 3–10 disaccharides (o-HA) (Al'Qteishat et al., 2006). In acute stroke patients, the plasma level of hyaluronan increases significantly (Tang et al., 2014). The plasma level of o-HA also increases for two weeks after ischemic stroke, with a peak at the seventh day (Al'Qteishat et al., 2006). The plasma level of o-HA exhibits a U-shaped association with clinical outcomes, indicating that both too-high and too-low hyaluronan levels may lead to unfavorable outcomes. By contrast, the plasma level of hyaluronan under 500 ng/mL 48 h after onset is an independent, favorable outcome predictor in intracerebral hemorrhage patients (Tang et al., 2014).

Stroke induces the expression of hyaluronan synthases and hyaluronidases. HAS1, HAS2, HYAL1, and HYAL2 are upregulated in inflammatory cells from both infarcted and peri-infarcted regions. HYAL1 is upregulated in microvessels and intracellularly in neurons, while HAS2 is translocated into the nuclei of neurons in peri-infarcted areas. Hyaluronidase activity in plasma also increases and peaks on the third day after stroke (Al'Qteishat et al., 2006). In a rat model of middle cerebral artery occlusion, HYAL1 and HYAL2 were upregulated for the first three weeks after stroke, with HYAL1 increasing earlier than HYAL2 (Al Qteishat et al., 2006). In a mouse model of intracerebral hemorrhage, we also observed upregulation of HAS1-3 and HYAL1-3 in areas receiving autologous blood injection (Ding et al., 2013). The loss of HAS1 function in mice appears to break this balance, aggravating apoptosis and necrosis (unpublished observations).

The above findings demonstrate that hyaluronan production and fragmentation is a feature of the acute stage of stroke. The production of o-HA may have

detrimental effects, since it enhances the inflammatory response. On the other hand, hyaluronan and/or o-HA may induce angiogenesis and revascularization, promoting the survival of at-risk neurons.

Stroke also causes the upregulation of HABPs, including CD44, RHAMM, and TSG-6 (Wang et al., 2001; Al'Qteishat et al., 2006). CD44 upregulation was observed in microglia, mononuclear cells/macrophages, and vascular endothelial cells in stroke-affected areas. Resident microglia and blood-derived macrophages are the major inflammatory cells activated by hyaluronan after cerebral ischemic events, but their sensitivities to extracellular mediators may differ (Wang et al., 2006). Hyaluronan induces a higher level of MAPK phosphorylation in RAW264.7 macrophages than it does in BV-2 microglia. In addition, the secretion of tumor necrosis factor α (TNF-α) is 5- to 10-fold greater in peritoneal macrophages and RAW264.7 than in primary microglia and BV-2, respectively (Wang et al., 2006).

Endogenous neurogenesis after stroke is insufficient to compensate for the damaged brain tissue, largely due to the lack of proper biological structure to establish new cells in the lesion area (Arvidsson et al., 2002; Doeppner and Hermann, 2015). Multiple studies show that scaffolds made of hyaluronan biomaterials help provide a suitable environment for not only new neurons, but also vessels, glia, and neurofilaments. In a model of stroke using athymic mice, implantation of hyaluronan biomaterials increased proliferation and neurogenesis in the subventricular zone ipsilateral to the ventricle (Sanchez-Rojas et al., 2018). Neuroblasts, glial cells, and endothelial cells forming capillaries were found inside the implant. These repairing events were further enhanced by simultaneous transplantation of adipose stem cells, which attenuated inflammatory reactions (Sanchez-Rojas et al., 2018). Tissue repair after stroke may also involve the activation of astrocytes by LMW hyaluronan–CD44 interaction, which induces Rac1-dependent protein kinase N-γ (PKNγ) activity, and subsequently upregulates the phosphorylation of the cytoskeletal protein cortactin, leading to the attenuation of cortactin-F-actin crosslinking (Bourguignon et al., 2007).

5.4 Other cerebral disorders

5.4.1 Multiple sclerosis

Hyaluronan accumulation in demyelinated lesions has been observed in multiple sclerosis patients and in mice with experimental autoimmune encephalomyelitis. HMW hyaluronan synthesized by astrocytes inhibited remyelination after lysolecithin-induced white matter demyelination, probably by preventing the maturation of oligodendrocyte progenitors that are recruited to demyelinating lesions (Back et al., 2005).

5.4.2 Epileptic seizures

Epilepsy is the second most common neurologic disorder next to stroke in China, yet it remains one of the least understood major chronic conditions. Among the three *Has*-deficient mice, namely $Has3^{-/-}$, $Has1^{-/-}$, and $Has2^{CKO}$, seizures are most prevalent in $Has3^{-/-}$ mice, which show the greatest reduction of hyaluronan in hippocampus (from 12% to 7%), and a selective reduction (40%) of ECS volume in stratum pyramidale (Arranz et al., 2014). Electrophysiology in $Has3^{-/-}$ brain slices demonstrated spontaneous epileptiform activity in CA1 pyramidal neurons. Histological analysis revealed an increase in cell-body density in the CA1 stratum pyramidale (Arranz et al., 2014).

5.4.3 Aging

The brain microvasculature is supported by the surrounding ECM, which is comprised mainly of hyaluronan. Studies in neonatal, adult, and elderly rats show that the density of cortical microvasculature decreases with age, while the perivascular hyaluronan level increases (Reed et al., 2017). There is no age-associated change in the hyaluronan molecular mass profile. Both *HAS2* mRNA and protein are observed to increase in aged microvasculature (Reed et al., 2017). By contrast, mRNA levels encoding hyaluronidases or HABPs relating to hyaluronan degradation do not change with age. Therefore, it seems likely that increased hyaluronan promotes the neuroinflammatory response, causing the decrease of density of microvascular system (Reed et al., 2017).

6 Conclusions

Hyaluronan is involved in most major cardiovascular and nervous system disorders. Its roles in these disorders are wide-ranging, depending on the location, type, and stage of lesion; in addition, the amount, molecular mass, and the presence of HABPs

all have a significant influence. The findings summarized here should help improve therapeutics for these disorders; for example, since hyaluronan plays key roles in plaque formation, the prevention or attenuation of atherosclerosis by manipulating hyaluronan will be an important research direction in the future. For stroke, there are still few effective treatments available, except early antithrombotic medications and thrombectomy. Recovery after stroke remains a baffling problem. Stem cell transplantation is one of the hotspots of current research for novel treatments, and hyaluronan and its binding proteins are known to assist the colonization and survival of stem cells. Therefore, it is important to include these molecules in the optimization of stem cell techniques.

Contributors

Hong-yan DING collected and analyzed information and drafted the manuscript. Ya-nan XIE and Qiang DONG collected and analyzed information. Koji KIMATA, Yoshihiro NISHIDA, and Naoki ISHIGURO provided general supports and reviewed the manuscript. Li-sheng ZHUO revised the manuscript. All authors have read and approved the final version.

Compliance with ethics guidelines

Hong-yan DING, Ya-nan XIE, Qiang DONG, Koji KIMATA, Yoshihiro NISHIDA, Naoki ISHIGURO, and Li-sheng ZHUO declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题　目：透明质酸在心血管和脑病变中的作用

目　的：透明质酸是细胞外基质的重要组成成分，不仅具有结构支撑功能，而且还积极参与调节细胞的功能。透明质酸的理化学和生物学特性受其自身的分子量大小以及数十种结合蛋白的影响，从而在众多的生理和病理过程中展现出多姿多彩的生物学功能。本文以心血管和脑病变为中心，总结透明质酸在这些病变的发生和修复过程中的作用，以期服务于今后的基础研究和临床实践。

关键词：透明质酸；细胞外基质；心血管；心脏；脑