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Property and current clinical applications of mammal hyaluronidase

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Abstract. – Hyaluronidase (Hyal), which is related to mammalian diseases, is greatly significant for mammal. It is the major enzyme for degrading hyaluronan (HA), which is a linear high molecular polymer that is ubiquitous in mammalian extracellular matrix. Previous studies suggested that the levels of Hyals play significant roles in predicting, determining and curing many diseases.

This review summarizes previous studies on the classification and biophysical and therapeutic applications of mammalian Hyals and focuses on the current medicinal and clinical applications of Hyals.

Key Words:
Mammal hyaluronidase, Medicinal and clinical applications, Tumor cells.

Introduction

Hyaluronidases (Hyals) are endo-β-N-acetylhexosaminidases (EC 3.2.1.35) that predominantly hydrolyze hyaluronan (hyaluronic acid or hyaluronate) and catalyse transglycosylation. They have limited ability to degrade chondroitin sulphate (C4-S, C6-S)[1-3]. Hyaluronan (HA) is a high-molecular-weight, unsulfated glycosaminoglycan that comprises repeating units of [N-acetylglucosamine (1-β-4) D-glucuronic acid (1-β-3)]$_n$ (Figure 1). HA dominates the macromolecular components of the extracellular matrix (ECM) in mammals; these components include connective tissues, eyes, skin, cartilage, aorta, synovial fluid and splanchnic nerves[4]. Mammalian Hyals can overcome the physical barrier that is formed by HA, and change the penetrability of ECM. They are ubiquitous in stroma and tissues from the liver, brain, testicle, plasma, and ECM[5]. Mammalian Hyals have attracted increasing attention because of their functions in several physiological and pathological reactions, such as fertilization[6], embryogenesis[7], wound healing[8], tumor growth[9] and tumor malignancy[10]. This review summarized the bio-physical and chemical properties of mammal Hyal as well as the medicinal and clinical applications of mammalian Hyals.

Mammalian Hyaluronidases

Mammalian Hyals are hyaluronan 4-glycanohydrolases, which degrade HA by cleaving the β-1,4-glycosidic bond and produce a tetrasaccharide molecule as their main product. They involve both hydrolytic and transglycosidase activities[11]. The degradation of mammalian Hyals follows a random biting-type catalytic method[12], which is an endo, non-progressive mechanism, in degrading products and substrates.

Genomic analysis reveals that mammals possess multiple Hyal genes. In humans, the *Hyal (hHyal) -1*, *hHyal-2* and *hHyal-3* genes are tightly clustered on chromosome 3p21.3 whereas the *hHyal-4*, *hHYALP1* (hyaluronidase pseudogene gene 1) and *hPH-20* are clustered on chromosome 7q31.3[5]. *HYALP1* is expressed as a pseudogene in humans[13] but encoded as an active enzyme in mice[14]. Meningioma expressed antigen-5 (MGEA5) is the eighth hyaluronidase-like protein coded for by *Mgea5* on chromosome 10q24.1-q24.3. This protein demonstrated a hyaluronidase activity and is identified as a novel immunogenic antigen that is expressed in meningiomas[15]. In mice, *Hyal-1*, *Hyal-2* and *Hyal-3* are located on chromosome 9F1, whereas *Hyal-4, HYALP1, PH-20* and *Hyal-5* are located on chromosome 6A[16]. However, the existence of Hyals provides vast potential for both constitutive and regulated turnover of HA[12,17]. All mammalian Hyals have different substrate specificities and expression profiles. The properties of these enzymes are listed in Table I.

Hyal-1 is a secreted Hyal that serves as the major type of Hyals in human plasma and is

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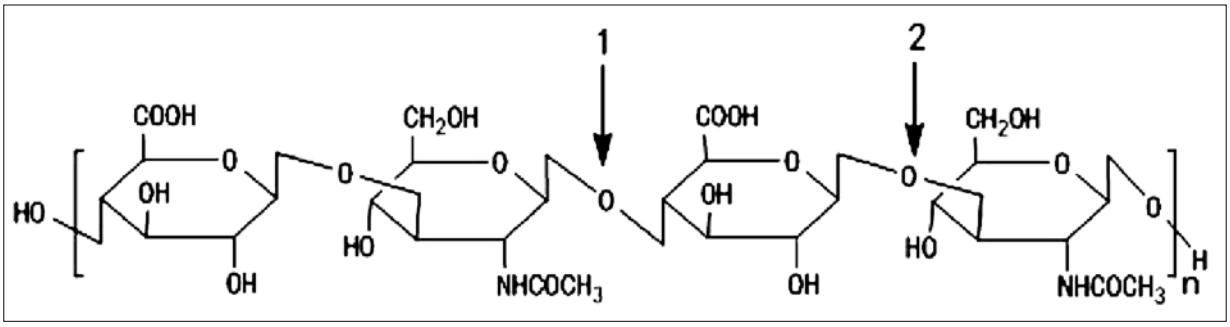

Figure 1. Structure of Hyaluronan. 1, β-1, 3 glycosidic bond; 2, β-1, 4 glycosidic bond.

ubiquitous in somatic tissues. Hyal-1 is responsible for the catabolism of HA in ECM. It is an acid pH optimum enzyme[11]. Hyal-1 is associated with many diseases, such as tumor growth, invasion and the deterioration. A deficiency in Hyal-1 causes a lysosomal storage disorder in humans called mucopolysaccharidosis IX[26]. The transformation of Hyal-1 in tissues is involved in the healing of damaged tissue and wound[27].

Hyal-2 is also found in most tissues, except in the adult brain. Hyal-2 has a lower activity than Hyal-1 and PH-20[20,28]. It is localized either in lysosome or is connected to the vascular membrane surface as a glycophosphatidylinositol (GPI)-anchored cell surface protein[29]. Hyal-2 also serves as the cell-entry receptor for Jaagsiekte sheep retrovirus which is associated with lung cancer[30]. Hyal-2 is also an acid pH optimum enzyme. It degrades high molecular weight HA into 20kDa HA fragment, which corresponds to 50-60 disaccharide units[20]. This HA fragment has distinctive biological function. In specific Hyal-2 can stimulate angiogenesis, which facilitates the growth of solid tumors[28]. However, the special function of Hyal-2 in mammals needs further investigation.

Hyal-3 is relatively unexplored as compared with other Hyals. This protein is highly expressed in testis and bone marrow, but is weakly expressed in other organs[5,31]. Hyal-3 is also expressed in the adult brain, in which Hyal-1 and Hyal-2 are rarely found. A functional study showed that mice without Hyal-3 were viable and fertile, exhibited no gross phenotypic change and showed no indication of HA accumulation[32]. Hyal-3 may not play a major role in HA degradation at least in mouse[32].

Unlike other Hyals, human hyal-4 is a chondroitin sulfate-specific endo-β-N-acetyl-galactosaminidase that exerts no hyaluronidase ac-

Table I. Properties of Hyaluronidases.

Type	Expressed sites	Molecular weight	Optima pH	Substrate	Product	Reference
Hyal-1	Widely expressed, (plasma) intracellular	435AA	3.0 -4.5	HA Ch/CS	–	18
Hyal -2	Widely expressed, extracellular	452AA	6.0-7.0	HA (Only high molecular weight HA	20kDa oligosaccharides	19, 20
Hyal -3	Testis, bone marrow, weak expression in other organs	45.0-56.0kDa	–	HA	–	21
Hyal -4	Restricted to placenta, skeletal muscle, testis	54.0kDa (481AA)	5.0	Ch	–	5, 22
HYALP1	All organs, an expressed pseudogene	–	–	–	–	5
PH-20	Testis extracellular	80.0-97.0 kDa 30-59kDa	4.0, 7.0	HA Ch/CS	–	23, 24
Hyal -5	Testis	–	–	HA	–	25
MGEA5	–	–	–	HA	–	15

tivity[3]. Hyal-4 is also a GPI-anchored chrondroitinase[3,22]. HYAPL1 has been detected translated in many human tissues, but does appear to be active. HYALP1 has a unique function in the adult testis and may compensate for the function of SPAM1 in *SpamI* null mice[13].

PH-20 (Sperm adhesion molecule 1, SPAM1 or PH-20) is the most active mammalian Hyal. It degrades HA into small fragments, mostly tetrasaccharides. PH-20 is a GPI-anchored protein only located on the plasma membrane of sperm. PH-20 is the major sperm Hyal and plays an important role in sperm adhesion to the oocyte zona pellucida and in sperm penetration through the cumulus matrix[33]. Sperm without PH-20 were present in the cumulus matrix at a low rate and the numbers of sperm were lower than normal at the early stages after insemination *in vitro*[34]. However, these proteins still had the ability to reach the oocyte zona pellucida and undergo fertilization[33]. PH-20 may not essential in fertilization in mouse[35], but it is still has an exact effect on fertilization.

Hyal-5 is also a GPI-anchored Hyal that contains epididymal sperm that can only be found in mouse testes. This protein helps PH-20 degrade the HA-rich matrix of the cumulus layer that surround the egg[33]. The lack of Hyal-5 in mice did not find significantly effect on fertilization. Therefore, Hyal-5 may be considered dispensable for fertilization[33]. MGEA5 is the eighth hyaluronidase-like protein coded by *Mgea5* on chromosome 10q24.1-q24.3. MGEA5 is a novel immunogenic antigen that is expressed in meningiomas and demonstrated Hyal activity[15].

Homology Analysis

Mammalian Hyals are widely distributed in mammals. Similarities in the amino acids sequences of mammalian Hyal were analyzed using DNAMAN Version 4.0 (Lynnon LLC., San Ramon, CA, USA). High similarity rates of 83.86%, 88.52%, 88.07%, 66.76%, 90.92%, and 91.72% were observed in Hyal-1, Hyal-2, Hyal-3, Hyal-4, PH-20, and MGEA5, respectively.

Phylogenetic analysis was performed using MEGA6.0. The amino acids sequences of the mammalian Hyals from *Homo sapiens, Rattus norvegicus, Mus musculus, Bos taurus, Sus scrofa* and *Pan troglodytes* were chosen to construct a phylogenetic tree. The phylogenetic analysis results in Figure 2 are consistent with the Hyal classification. Mammalian Hyals were divided into seven subgroups. MGAE5 achieved the lowest similarity with the six other mammalian Hyals. Hyal-1, Hyal-2 and Hyal-3, which were clustered on the same choromosome, had a near-similar homology. PH-20 and Hyal-5, which were expressed in the sperm, also had a near-similar homology. Hyal-4 had a higher similarity with PH-20 and Hyal-5 than with Hyal-1, Hyal-2 and Hyal-3. These subgroups of Hyals were closely associated with their locations on chromosomes and their functions. This homology indicated an ancient gene duplication, which could motivate future studies on the different Hyal subtypes with unknown functions.

Function of Mammalian Hyaluronidases in Cancer Biology

The effect of the ECM degrading enzyme Hyals on interstitial fluid pressure and microvascular pressure was measured in many human tumors[36]. The levels of Hyals and HA are closely associated with many types of tumor cell lines[37,38]. The Hyals that are expressed by tumor cells degrade HA to small HA fragments of 2 to 15 disaccharide units which can stimulate angiogenesis, and initiate endothelial cell proliferation via the cell surface receptors (RHAMM and CD44)[39]. HA-CD44 interactions are important in malignant tumor growth angiogenesis induction and tumor progression *in vivo*[10]. Blocking Hyal-1 expression in invasive bladder cancer (BCa) cells decreases tumor growth, inhibits tumor infiltration, and decreases microvessel density[29]. Adding Hyal-1 and Hyal-2 in a culture dose not inhibit tumor growth *in vitro*. The overexpression of Hyal-2 not only increases the growth rate of tumor cells but also enhances the invasiveness and vascularization in intracerebral glioma[37].

Some studies suggested that Hyals exhibited a very strong inhibiting effect[10,40]. In human genomes, Hyals genes clustered on chromosome 3p21.2 were considered as conserved candidate tumor suppressors[16]. Wang et al[40] found that Hyal-2 showed some tumor suppressor activity in SCID mice. Chang et al[41] reported that Hyal-1 and Hyal-2 increased the susceptibility of tumor necrosis factor in L929 cells. This study proved that the transforming growth factor β 1 only promoted the growth of L929 cells but not the growth of Hyal-2 and Hyal-2-expressing L929 cells[42]. The use of Hyals can enhance the action of immune effector cells via degradation of the HA-formed halos around cancer cells that look similar to adherent fibrosarcoma cells[43].

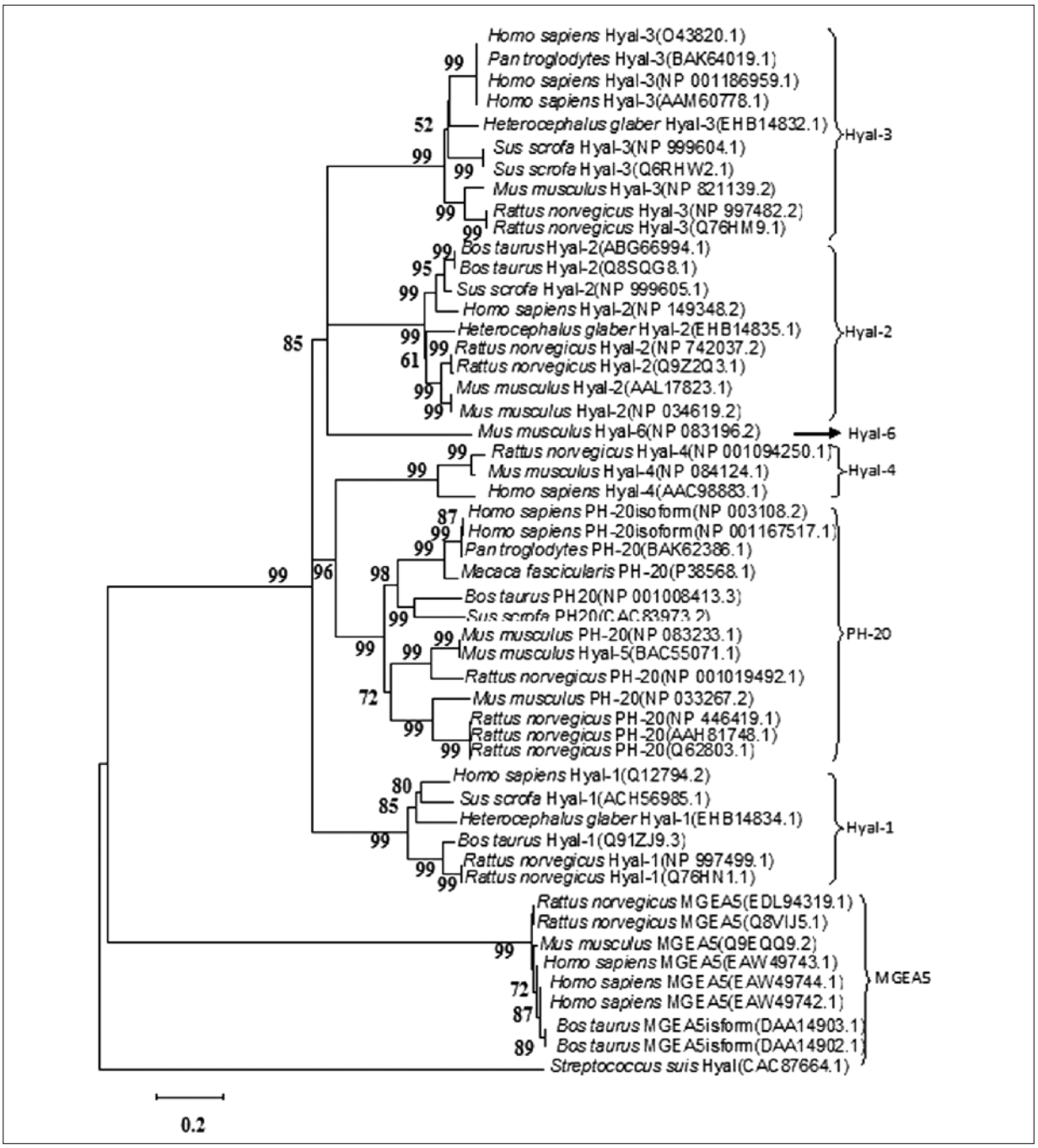

Figure 2. Phylogenetic trees showing the affiliations of Hyal amino acid sequences (GenBank number). The tree was constructed by neighbor-joining using full-length aligned amino acids sequences with *Streptococcus suis* as an outgroup. The bootstrap value is based on 1000 replicates and is shown at the nodes with more than 50% bootstrap support. The scale bar represents 20% sequence divergence.

However, some studies suggested that Hyals exhibited both inhibiting and facilitating effects on cancer. This finding is closely related to the cellular concentration of Hyals[18]. Hyal variants were also found in tumor cells. For example, Hyal-1 variants were found in tumor cells of BCa[44]. Eleven types of Hyal-wild (1, 2, 3) or variants were found in adenocarcinomas and squamous cell[45]. Previous studies concluded that the function of Hyals in normal cells and their variants in tumor cells remains unclear.

Although the function of Hyals in cancers has yet been clarified, they can be used as markers

and therapeutic adjuncts in cancer. Hyals can also be used as a non-invasion method for determining cancer prognosis. Eissa et al[46] showed that the measuring Hyal-1 mRNA by semiquantitative PCR can be performed to diagnoses BCa. Moreover, Hyal achieves high accuracy in detecting cancer and evaluating its grade[46]. Hyals can also serve as markers to accurately detect early and high-grade BCa as well as to predict prostate cancer progression. Virotherapy, studies suggested that oncolytic adenoviruses that expressing PH-20 have great antitumor efficacy after systemic administration in virotherapy for cancer. The co-application of Hyals with oncolytic adenovirus can enhance the anti-cancer activity[47, 48]. When chemically treatment of malignant tumors, chemotherapy drugs that are leaked into the surrounding tissue or intravenous injection of such drugs into the extravasation accident can damage tissues, nerve and tendons. Hyals can reduce intratumoral pressure, which allows the drug to penetration into the malignant tumor and relieve the chemical drug out of vessels[49].

Clinical Application and Prospect of Mammalian Hyaluronidases

The biological function and therapeutic applications of Hyals have recently attracted considerable academic attention. Hyals have potential application in postoperative operation, giving of macromolecular drugs, trauma treatment and wound healing[50].

Hyaluronidases as Adjuvant

Mammalian Hyals have been widely used as adjuvants in clinical settings to administer local anesthetics[51,52], replacement fluid[53], insulin[54], corticosteroid and large molecules[55]. Hyal can reduce the viscosity of ECM. A lesser viscosity of ECM facilitates the diffusion of the anesthetic agent. Adding Hyals with lidocaine can enlarge the anesthetized area, shorten the drug diffusion time, increase the permeability of tissues, and enhance the anesthesia effect[56]. Therefore, the use of Hyals in cardiac patients is beneficial because they strengthen the anesthetic effect and decrease the volume of applied anesthetic. Hyals can also effectively improve the absorption of X-ray opaque at drugs. Injecting Hyals has also been considered safe agent without any combined applications[51]. Recombinant human Hyals (rHuHyals) has advantage of high purity, favorable efficacy and safety[57]. Hyals may be suitable alternatives for clinical use than compounded animal-derived products[58].

Hyaluronidases in Ophthalmology

Hyals have been used in ophthalmology for many years[59]. Hyals can be injected with anaesthetic for retrobulbar, peribulbar and sub-Tenon's blocks[59-61]. They can promote the spread of the anaesthetic (bupivacaine or lidocaine or ropivacaine et al[62]) through the tissues or (and) reduce the volumes of local anaesthesia[60]. It was found that Hyals can be used for pharmacologic vitreolysis to avoid the complication of surgery such as cataract, endophthalmitis and anesthesia related complications[63]. The advantages of using Hyal also include enhancing speed of onset and improving operating conditions[64].

Previous studies[58,62,65] indicated that the purity and optimal dosage of Hyals were crucial in ophthalmology. Important factors that influence the clinic use of Hyals still need investigation, such as injection site and techniques, the choice of anaesthetic solution and the optimal dosage[58,62]. In addition, the toxicity of Hyals still needs further studies[61,66,67].

Hyaluronidases in cancer forecast and Therapy

Elevated levels of Hyals and HA in plasma and urine have been reported to be associated with the presence of tumors, like BCa[68], epithelial ovarian cancer[38], lung cancer, prostate cancer, and breast cancer[69]. Therefore, Hyals may have potential advantages for tumor diagnosis[46]. Jamshldlan et al[70] devised a highly accurate and non-invasive cancer diagnosis method that involves measuring the urinary levels of HA and Hyals and evaluating its grade in BCa. Hyal-1 is expressed in bladder cancer lines and serves as an accurate marker for high-level bladder cancer.

Hyal can be used to treat intravenous extravasations. They can also be used as antidote to the extravasation of vinca alkaloids; this condition results from the complication of severe tissue necrosis during administration[49]. The absorption of Hyals can also prevent local necrosis from exuding further. The subcutaneous administration of Ondansetron with rHuHyals is generally tolerated in minipigs and healthy volunteers[71].

Hyaluronidases in Plastic Surgery

Hyals are commonly applied in plastic surgery to reverse the effects of fillers overdose, eliminate nodules, and reduce the odds of ischemic complications. Hyals can also reduce skin damage and prevent postoperative wounds from swelling[72].

HA fillers are widely used in plastic surgery. However, these fillers can cause several complications that range from superficial placement, uneven placement, granulomatous reactions, and skin necrosis[73]. Hyals can effectively solve such problems. The rapid response of Hyals is particularly helpful in compromise skin malposition or overfill. Given their safety and effectiveness, Hyals are generally used as HA filler to prevent severe complications[74]. Several experiments have proven that infiltration of Hyals can rapidly, effectually, and safely solve eyelid edema following the injections of HA fillers[58]. Cavallini et al[75] reported that ovine Hyal dosages below 14U did not affect fibroblast viability.

Hyaluronidases in Fertilization

PH-20 is the major type of Hyal in testis that significantly increases the ability of null sperm to penetrate the cumulus of oocytes. The relation between PH-20 and fertilizing ability indicates that PH-20 is a marker of sperm maturation[76]. Although some experiments have shown that PH-20 null mice are still fertile, their fertilization efficiency has significantly decreased[34,77]. Asides from these functions, PH-20 also has high contraceptive properties because of its strong immunogenicity and essential role in fertilization[78]. Hyal-1 and Hyal-3 also play important roles in ovarian folliculogenesis and female fertility[79].

Hyaluronidases in Immunoreaction

Combining human immunoglobulin with rHuPH-20 offers a new method for subcutaneous delivery of human immunoglobulin replacement therapy in patients with primary immunodeficiency disorders. rHuPH-20 increases the volumes of infused subcutaneous immunoglobulin and facilitates the absorption immunoglobulin in the lymphatic, particularly in pediatric population[80,81]. This method is equally effective in the administration of intravenous immunoglobulin and induces fewer complications[55]. This novel method of delivering immunoglobulin is considered safe, effective and well tolerated by patients. It still needs more data to declare the long-term safety[55].

Potential Functions

Hyals have potential applications in the DNA vaccine. Hyals can also improve conversion efficiency and promote antibody expression during skeletal muscle inflammation treatment among mice via DNA vaccine. Previous studies have also indicated that Hyal improved the initial complement of interleukin (1L)-1β expression, cancer cell necrosis factor α, interleukin-6 cytokine in inflammatory cells, thereby enhancing the power transfer effect.

Conclusions

Mammalian Hyals has a lots of important physiological processes. However, compared with the Hyals from bacteria or venom, those from mammals remain unexplored in terms of their structure and metabolic process in HA function[82]. Understanding the mechanism of Hyals in cancer progression may be useful for future cancer prognosis and may offer novel pharmacological opportunities for cancer treatment. A guideline must also be devised for using Hyals in clinical application, particularly on their optimal dosage and treatment[83]. Future work on mammalian Hyals and their clinical use must: (1) elucidate the relationship between structure and function, (2) reveal the mechanism of Hyals in related diseases, (3) develop genetic methods for the rapid production of safe and long-term Hyals, (4) establish the assay of using Hyals and the appropriate dosage.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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