Lamprey: a model for vertebrate evolutionary research

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ABSTRACT

Lampreys belong to the superclass Cyclostomata and represent the most ancient group of vertebrates. Existing for over 360 million years, they are known as living fossils due to their many evolutionally conserved features. They are not only a keystone species for studying the origin and evolution of vertebrates, but also one of the best models for researching vertebrate embryonic development and organ differentiation. From the perspective of genetic information, the lamprey genome remains primitive compared with that of other higher vertebrates, and possesses abundant functional genes. Through scientific and technological progress, scientists have conducted in-depth studies on the nervous, endocrine, and immune systems of lampreys. Such research has significance for understanding and revealing the origin and evolution of vertebrates, and could contribute to a greater understanding of human diseases and treatments. This review presents the current progress and significance of lamprey research.

Keywords: Lamprey; Nerve; Endocrine; Immune; Functional gene

INTRODUCTION

Lampreys have seven separated gill holes aligned behind the eyes on both sides of the head and appear like eight eyes. Lampreys pertain to Cyclostomes, rather than Osteichthyes (Kuratani et al., 2002), and are the most primitive agnathans (jawless fish) among marine animals, belonging to Cyclostomata, Vertebrata, Chordata. Vertebrates are divided into the evolutionarily more advanced gnathostomes and the inferior agnathans, in which Cyclostomes predominate (Figure 1) (Janvier, 2006). The existing Cyclostomata contain two categories: Petromyzonida and Myxini. The Petromyzonida contain Petromyzon in North America, Mordacia in the Southern Hemisphere, and Lampetra in China. Lampetra include: *Lampetra japonica*, *Lampetra morii* and *Lampetra reissneri*, among which *Petromyrzon marinus* spread to the Great Lakes region, and *Lampetra japonica* and *Lampetra morii* to China (Freamat & Sower, 2013; Shimeld & Donoghue, 2012).

Cyclostomata first appeared in the Ordovician, peaked during the Silurian and Devonian, and then experienced gradual extinction in the late Devonian. The piecemeal discovery of fossils has proven that lampreys existed 360 million years ago, before the time of dinosaurs, and their shape has stayed almost unchanged over hundreds of millions of years of evolution. Lampreys are, therefore, universally termed as "living fossils" due to their strong resemblance to early fossil material, which reflects their important position in vertebrate evolution (Gess et al., 2006).

Lampreys live in rivers and oceans, and most are migratory parasitic. They repeat the cycle of reproduction and death like other anadromous fish. The larvae (ammocoetes) spend the first 7-9 years of their life in freshwater streams, after which they undergo metamorphosis and develop suctorial discs, eyes, and dorsal fins, and then migrate to the ocean. When lampreys reach full adult size via feeding on fish blood, they become sexually mature and migrate back to the streams and rivers (Youson & Sower, 2001).

Rats, mice, *Xenopus*, zebra fish, globefish, *Caenorhabditis elegans* and *Drosophila* are well-established model organisms, with extensive genome sequencing and scientific studies on such species greatly contributing to the health and treatment of human diseases (Fuller & Tomé, 2005). Considering their special status in vertebrate evolution, studies on lamprey species have attracted the growing attention of biologists worldwide. With the development of modern molecular techniques, lampreys have become one of the most important research models for understanding vertebrates. The following review discusses current research on the nervous, endocrine, and immune systems of lampreys.

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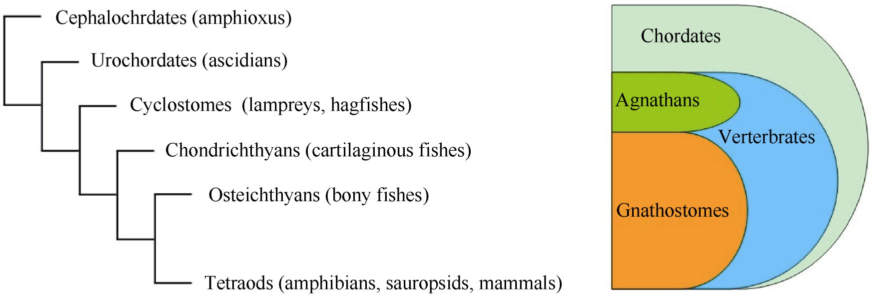

Figure 1 Phylogeny of select Chordate taxa

Labels on the right indicate the names of the monophyletic groupings shown on the left.

PRIMITIVE FEATURES AND ORGANS OF LAMPREYS

In marine organisms, lampreys are the most primitive extant vertebrates, with the earliest fossil record tracing back to the Ordovician. Lamprey research began in the mid-19th century. At that time, lampreys were considered as direct ancestors of modern vertebrates. Many initial studies focused on comparing the morphological differences between adult lampreys and higher vertebrates to ascertain their taxonomic positions. The lampreys used for those studies were all obtained from the wild, with ammocoetes were first captured in 1900 (Reese, 1900). With the establishment of *in vitro* fertilization techniques and short-term feeding, it became possible to study lamprey embryonic development. Embryonic development of sea lamprey was first described by George Piavis (Piavis, 1961), with his developmental staging system still widely used today (Nikitina et al., 2009).

The appearance characteristics of lampreys are very primitive, and their tissues and organs can represent the initial state of vertebrate evolution and development. They do not have jaws for predation, and possess median rather than paired fins, which are sustained by unsegmented ray-finned cartilages. Furthermore, they reserve notochords to support their body axis. Their skulls are incomplete, consisting of only a joint of ear cartilages between both sides of the ear cartilage capsules, and a fibrous tissue membrane over the brain, similar to the skulls of higher vertebrates at the early stage of embryonic development. The gill cage is composed of gill arches and cartilages, and is responsible for supporting respiration. The terminal of the gill cage is sealed with pericardial cartilages to protect the heart. The bodies and tail muscles of lampreys are also the most primitive bow sarcomere of all vertebrates. Their digestive systems have not differentiated into stomachs. The blind ditches (the original morphology of intestinal villi) are differentiated in the intestinal tubes, and the real liver appears. The heart, which consists of the sinus, one atrium, and one ventricle, appeared in the circulation system of lampreys The circulation system is a closed tube type like that of higher vertebrates, whereby blood flows in closed vessels. Each part of the lamprey brain is arranged in the same plane, without any brain partitioning. The mesencephalon does not form a bigeminum, and the cerebellum and medulla are not separated. The optic nerve does not develop into an optic chiasma, and there are only one or two semicircular canals in the inner ear balancer (Janvier, 2008; Kuratani, 2005).

Considering the features mentioned above, lampreys show extraordinarily high primitiveness, indicating that they are situated at the lowest level of vertebrate evolution. Research on comparative anatomy and embryology reveal that all chordates have three main features in common at a certain stage of their ontogeny or whole life, that is, the dorsal tube, notochord, and pharyngeal gill slits. This phenomenon indicates that all chordates originated from a common ancestor. As a consequence, lampreys are not only pivotal species for studying the origin and evolution of vertebrates, but also the best model for investigating vertebrate embryonic development and organ differentiation.

LOCOMOTOR BEHAVIOR AND NEURONAL NETWORK OF LAMPREYS

Nerve cells play an important role in an organism's behavior. Although studies on various ion channels at the single-cell level have achieved rapid development, exploration of the relationship between disparate locomotor behaviors and the entire neural network operation still face many difficulties. Drawing a complex circuit diagram of a neural network remains challenging, and the monolithic operation mode of the nervous system is hard to decipher with current technology. Therefore, the best way to explore such issues is to use vertebrates containing simple neural networks. The number of lamprey nerves is relatively small, with just one brain and one spinal cord, which makes studying their motion relatively easy. Hence, lampreys are widely used for studying the mechanism of swimming controlled by neural networks. The brephic brain structures of different vertebrates show high similarity, whereas the adult stage structures exhibit great differences. Thus, choosing a brephic brain from a representative animal as experimental material can effectively clarify the processes of phylogeny and ontogeny of all vertebrate brains. In the evolution of chordates, the basic structure of the brain has not appeared in amphioxi, a close relative of vertebrates. Instead, amphioxi have cerebral vesicles, which are slightly dilated lumens at the front of the neural tubes (Holland & Holland, 2001). The brains of lampreys already possess the basic structures of the vertebrate brain, which are divided into the forebrain, diencephalon, midbrain, cerebellum, and medulla (Murakami & Kuratani, 2008). In embryonic development, biological ontogeny will repeat its phylogeny, which means that the development process of animal embryos usually repeats the important stages of germline evolution. The ontogeny of lamprey brains indeed replays the phylogeny of vertebrate brains. The structure of the lamprey brain can reflect the elaborate structural changes of higher vertebrate brains. Thus, as lower vertebrates, lampreys are one of the most valuable animal models for the study of vertebrate brains.

Lampreys have many visible neurons, making them highly suitable for microelectrode experiments. Moreover, their neurons can survive for a long time *in-vitro* in separation devices, which is helpful for bionics (Murakami & Watanabe, 2009). Professor Grillner and colleagues (Biró et al., 2008; Ménard & Grillner, 2008; Saitoh et al., 2007) conducted extensive in-depth work on the mechanism of neural network control of lamprey movements. They described the specific structure of the nervous system, and provided a theoretical description of the activity patterns of neurons in the network structure. They emulated the swimming motion characteristics of lampreys by utilizing neuronal activity described with the Hodgkin-Huxley (HH) model, which contained ion channels and included 3D models to simulate forward and backward swimming motion and changes in swimming direction. Kamali et al. (2013) constructed an experiment in which a simulated lamprey swam around a virtual environment containing visually detectable objects, which addressed mechanisms for the generation and selection of visual behaviors in anamniotes. Ericsson et al. regulated dopamine levels to study how the strength of synaptic connections affected mode movements in lampreys (Ericsson et al., 2013). Researchers have also investigated the effects on lamprey movement rhythm when their cephalic neuromeres and spinal neurons suffer from external stimuli (Cinelli et al., 2014; Kinkead, 2009).

Rétaux et al. (Rétaux & Kano, 2010) studied Sonic Hedgehog/Hedgehog (Shh/Hh) signaling in lampreys, and discovered that embryonic midline signaling is the driving force of vertebrate forebrain evolution. As the most primitive vertebrate, the neural crests and structures of lampreys are differentiated from the neural crests of jawed vertebrates, although they still lack some important neural crest derivatives, such as sympathetic ganglions. Research further suggests that the migration path of lamprey neural crest cells is similar to that of higher vertebrates.

These studies again show that lampreys are excellent animal models for studying the evolution of the vertebrate nervous system. The nervous system, neural synapses, and synaptic neurotransmitters of lampreys represent the most primitive state in vertebrates. Uncovering the mysteries of the human brain is a common goal of neurobiologists worldwide. Lampreys, as the headwaters of vertebrate evolution, might hold the key to unlocking human cognition and the brain maze map.

LAMPREY ENDOCRINE SYSTEM

Over the past decade, molecular genetics have developed rapidly. Gonadotropin releasing hormone (GnRH) is a key signaling molecule of the hypothalamic-pituitary-gonadal axis. In lampreys, olfactory stimuli may play a major role in regulating GnRH secretion. Research shows that GnRH in lampreys is divided into GnRH-I, GnRH-II, and GnRH-III, and is mainly distributed in the hypothalamus, where it controls the pituitary gland and thus plays an important role in gonadal development and maturation of gametes (Decatur et al., 2013). The lamprey endocrine system also plays a part in metamorphosis. In the initial metamorphosis stage of parasitic and non-parasitic lampreys, the levels of thyroid hormone and liothyronine in serum drop sharply (Freamat & Sower, 2010). In fact, the compounds that can affect thyroid hormone synthesis, such as $KClO_4$ and goitrogens, are capable of initiating metamorphosis in lampreys, and also lowering the level of serum thyroid simultaneously (Manzon & Youson, 1999). Sexual maturity in lampreys is slow, which does impact its use as a model organism. It is not only beneficial for lamprey research, but also helpful in finding therapies for human diseases caused by hormonal disorders if manual methods to accelerate the lamprey maturation process are used.

The hypothalamus of higher vertebrates produces corticotrophin-releasing hormone (CRH) under stress. This hormone can stimulate the anterior pituitary cells to release adrenocorticotropic hormone (ACTH), with adrenal glands then induced to release cortisol or corticosterone. However, there are no such steroid substances in lampreys. Roberts et al. (2014) confirmed that there was one potential corticosteroid, 17, 21-dihydroxypregn-4-ene, 3, 20-one, that participates in the endocrine regulation of compression resistance in lampreys. The mode of endocrine changes in lampreys under stress might be the prototype of how the endocrine system varies in higher vertebrates when they face ambient stresses.

It is not only advantageous for scientific research to find a way in which to promote the growth of lampreys, but studies on their endocrine systems can also help capture the lampreys which usually cause fishery damages. Due to their parasitic habits, lampreys can have considerable impact on fisheries. Fish farmers will disrupt lamprey breeding, development, and migration in order to prevent damage to their fisheries. For example, the use of the drug 3-trifluoromethyl-4-nitrophenol (TFM) can kill lamprey larvae, while avoiding other fish and organisms (Birceanu et al., 2014). Because of the high cost of TFM, however, it is imperative that new drugs are developed to mitigate lamprey impact. Lamprey hormones might be a suitable choice, whereby mature lampreys can be prevented from breeding by using specific hormones or derivatives to lure them to a predetermined fishing spot.

The lamprey endocrine system is simpler than that of humans, and the endocrine organs and related molecules are more primordial. Comparing the histology and biochemistry of the primitive lamprey endocrine system with the well-differentiated human endocrine system has enabled researchers to better comprehend the evolution of the vertebrate endocrine system.

LAMPREY IMMUNE SYSTEM

The origin and evolution of the adaptive immune system (AIS) remains an important field in immunological research. Jawless vertebrates are generally considered to have evolved the AIS. Therefore, clarifying the adaptive immunity of Agnatha is of great significance in revealing the origin and evolution of the AIS. Early experiments showed that lampreys could produce specific agglutinin memorially contraposing particulate antigens, such as anthrax and sheep blood cells (Fujii et al., 1979), indicating that lampreys should have an AIS. Initially, however, T-cell receptors (TCR), B-cell receptors (BCR), and major histocompatibility complexes (MHC), which are symbolic molecules of the AIS, could not be tested in lampreys and was not clarified until an alternative AIS set was found (Cooper & Alder, 2006).

Adaptive immunity relies on the formation of lymphocytes. Scientists have found lymphocyte-like cells in lampreys that are similar to the lymphocytes in higher vertebrates. Shintani et al. (2000) found homologous genes of PU.1/Spi-B (purine box 1/spleen focus-forming virus integration B), a gene involved in the differentiation of lymphocytes in jawed vertebrates, expressed in the lymphocyte-like cells of the digestive tract and inexplicably the ovary in lampreys. Thus, these results validated the existence of lymphocytes in the jawless vertebrates.

To find the molecules that mediate the immunoreaction of jawless animals, Pancer et al. (2004) identified a large number of cDNA clones encoding multiple leucine-rich repeat (LRR) modules, and instead of the V (D) JC composition of immunoglobulin-based BCRs and TCRs observed in jawed vertebrates, variable lymphocyte receptors (VLRs) of jawless vertebrates were found. VLRs are composed of variable LRR sequences, and are produced by novel molecular rearrangement. The germline VLR gene does not have an intact structure to encode any proteins. Many LRR-encoding cassettes with highly diverse sequences are adjacent to this gene. During the development of lymphocyte-like cells, these LRR-encoding cassettes are chosen randomly and sequentially incorporated into the germline VLR gene. Not only is the sequence of each LRR cassette diversified, but the number of cassettes inserted into the VLR gene is variable (1-9). Thus, a computational assessment of mature VLR gene sequences and LRR cassettes used to assemble them can predict a potential repertoire, which is almost equivalent to that of Igs (>10^{14}). Such diversity is enough to identify any antigen outside (Hirano et al., 2011) (Figure 2).

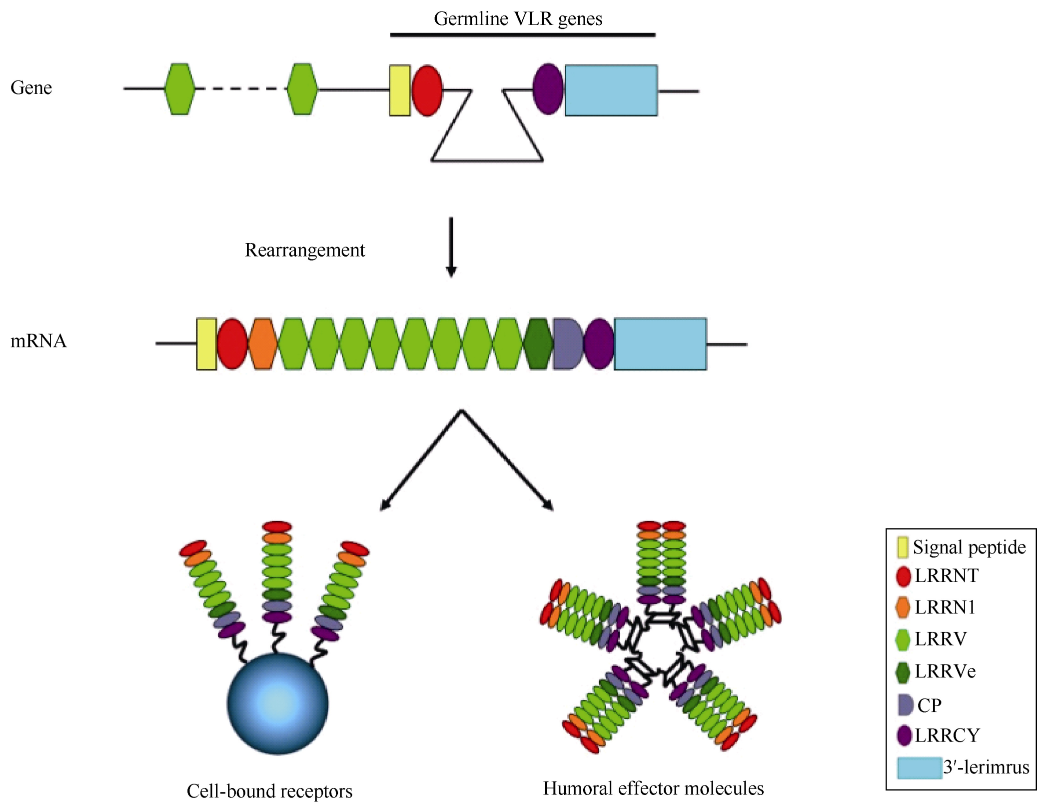

Figure 2 Rearrangement of antigen receptors of lampreys

In the vicinity of the incomplete germline VLR gene are multiple LRR-encoding cassettes. During the development of lymphocyte-like cells, these cassettes are sequentially incorporated into the VLR gene. LRR1, the first 18-residue LRRs; LRRV, 24-residue LRRs, whose number is variable; and LRRVe, the terminal 24-residue LRR with a distinct sequence signature.

Monoclonal antibodies have been widely used in research and clinical practices. However, immune globulins are costly, the manufacturing techniques are intricate, and the physicochemical properties are unstable. These defects have prompted research on finding alternatives. The discovery of VLRs affords a new perspective for the development of antibody reagents or drugs. The molecular weights of VLRs are about 15-45×10^3, while the relative molecular mass of IgGs can reach 156×10^3. Small molecular weights are more suitable for *in-vivo* transport of drugs and penetration into blood vessels, which means that VLRs are more applicable for the development of antibody drugs (Boehm et al., 2012). The monoclonal VLRs prepared by Herrin et al. (2008) had 1 000-fold higher affinity than IgGs at the same concentration, and could only be eluted under alkaline conditions (pH>11). In addition, the relationship between lampreys and humans is not close, so VLRs have good specificity as diagnostic reagents, eliminating cross-reactivities. The molecular structure of VLRs is relatively simple, with only one peptide chain. Compared with the hybridoma techniques of preparing monoclonal antibodies, the manufacturing techniques of VLRs are easier and production costs are lower, which makes it convenient for large-scale industrial production.

The discovery of immune organs is still a hot topic in lamprey research. As confirmed recently, lampreys do not have a thymus, lymph nodes, spleens, or tonsils, nor do they develop any specific immune organs and tissues. However, they must possess a powerful immune function to have withstood various environments and the invasion of germs over hundreds of millions of years. Accordingly, lampreys must contain a powerful immune organ to support the immune function. In lamprey larvae, researchers have found that lymphocytes might arise in the typhlosoles (i.e., sunken area of the intestinal epithelium) and renal folds (Bajoghli et al., 2011). However, information on the exact immune organs of adult lampreys remains uncertain. Recent lamprey studies by our group found a strip of medullary tissue throughout their entire trunk and tail above the nerviduct (Pang et al., 2015). It was preliminarily confirmed that this tissue was mainly made up of fat cells, hematopoietic stem cells, and lymphocyte-like cells. We termed it the “immune body” due to its potential immune function (Figure 3). In the cDNA library of the immune body constructed by our group, we found abundant immune molecules that still require further study. It is possible that a very large immune organ, together with substantial immune molecules, has allowed lampreys to survive till the present day during despite the impact of environmental migration and bacterial infection during evolution.

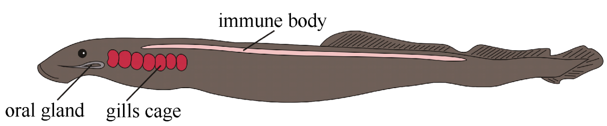

Figure 3 Location of immune body, oral gland and gill cage of lamprey

It has been a widely held belief that adaptive immunity in higher vertebrates evolved from the innate immune system (Du Pasquier, 2005). However, the discovery of VLR and its restructuring mechanism in jawless vertebrates impacted the innate immune evolutionary theory. This type of LRR-insert restructuring mechanism is likely the evolutionary origin of adaptive immunity. Currently, human immune-related diseases have become the culprits of health injury, even death. The in-depth exploration of the lamprey immune system is necessary for defeating serious human illnesses such as cancer, AIDS, and autoimmune diseases.

GENOME AND FUNCTIONAL GENES OF LAMPREYS

Since lampreys are living fossils of ancient animals, the lamprey genome could be described as a molecular fossil of ancient genetic information. Therefore, study of the lamprey genome could help clarify the origin and evolution of vertebrates. The lamprey genome is a record of the genetic information of vertebrates hundreds of millions of years ago, and exhibits many novel genes and proteins. Potter et al. (1968) discovered that chromosome number showed great variation between different sub-species of lamprey, 2*n*= 60-168. Because the number of lamprey chromosomes is large and the volume of each chromosome is very small, karyotype analysis can be difficult. However, it does provide some evidence for the chromosome doubling theory of vertebrates. The above phenomenon illustrates that the lamprey genome still remains in an intermediate stage of evolution from invertebrates to vertebrates.

Over the years, studies on lampreys have been confined to ecology and physiology, while genome and proteomic research remains scarce, especially for some important functional genes. Our group has conducted extensive lamprey genomic and proteomic research. We successively constructed lamprey cDNA libraries of salivary glands, liver, blood and immune body and other tissues, and sought out many novel genes and proteins with important physiological functions. For example, we found a Bruton's tyrosine kinase like (BTK) gene expressed in lamprey immune-related tissues, which might be involved in the immune response of lampreys (Wu et al., 2012). Reactive oxygen species modulator I (Romo I) and Peroxiredoxin (Prx) homolog were discovered, and are capable of inducing the production of reactive oxygen, resisting peroxidation of erythrocytes, and adjusting the cellular oxidation-deoxidation environment (Sun et al., 2010; Zhao et al., 2013). Transcription factor NFκB inhibitor I-κB-ε was also found in lampreys, and its expression was reduced under the stimulation of LPS (Su et al., 2013). Among the many functional genes found, the genes and proteins in the oral gland are special. In the evolutionary process, lamprey secreta could prevent blood coagulation of host wounds. Lampreys have a special way of life, which results in many proteins and genes with distinctive functions. The oral gland is wrapped in a special colored sac in the hypobranchial musculus (Figure 3), and can secrete an eel plasmin substance to prevent blood from clotting. We also found other functional proteins in lamprey oral glands, including those containing RGD (Arg-Gly-Asp) motif disintegrin-like proteins, retinoic acid, and interferon-induced lethality protein-19 (GRINM-19), tumor translationally controlled protein (TCTP), and gelsolin-like protein (Sun et al., 2008; Wang et al., 2010; Xiao et al., 2012). In subsequent future studies, we are hopeful to exploit intravenous, oral, or gene therapy drugs used for anti-tumor, anti-thrombotic, anti-virus, hypoglycemic, analgesic, and anti-heart failure treatment on the basis of these special function proteins.

Studies on lamprey functional genes can help to understand the detailed biological evolutionary process of different genes, and can also contribute to finding associated proteins or peptide drugs, which might be favorable for human disease therapy. Therefore, whether from the perspective of scientific research or application, studying the function of lamprey genes has far-reaching significance.

CONCLUSIONS AND PERSPECTIVES

Due to their unique way of life and appearance, lamprey research began as early as the 19^{th} century. Many studies focused on comparative biology, especially in the field of early vertebrate evolution. Lampreys have also been used in the study of evolutionary embryology and developmental biology. With the emergence of modern molecular techniques, lampreys have become one of the most important models for the exploration of vertebrate origins.

Ancient lamprey fossils have provided evidence of their emergence more than 360 million years ago. Their external morphology shows no obvious differences compared with existing lampreys, which is known as "evolutionary diapause". Lampreys exhibit primitive external morphology and physiology structure: no paired appendages; only horny teeth formed by the epidermis; notochord retained throughout life; skull incomplete, without top, equivalent to the early stages of embryonic development of the vertebrate skull; less muscle differentiation, original arrangement of sarcomeres; lower degree of brain development layer, and two semicircular canals. These original characteristics suggest that lampreys are the evolutionary node from invertebrates to vertebrates. Thus, due to their critical species status, lamprey research is fundamental to biological evolution and genomic studies.

The rapid development of cell biology and biochemistry technology, especially genomics and proteomics, has pushed lamprey research to new heights. Lampreys have a powerful and unique immune system and many valuable genes. Based on lamprey genome research, important human disease-related genes might be discovered. In addition, the pathogenesis of human diseases can be revealed by establishing lamprey genetic models of human diseases, which could provide the theoretical basis for in-depth study of the pathogenesis and treatment of diseases and development of new drugs. In recent years, lamprey numbers have drastically reduced, so lamprey research is important.

As a model animal for the evolution and development of vertebrates, lampreys have high research value due to their connection to vertebrates and invertebrates in evolution. Lampreys are not only key species for the exploration of the origin and evolution of vertebrates, but also the best model for studying vertebrate embryogenesis and organ differentiation. The construction of lamprey models will occupy a central position in vertebrate evolution and developmental studies during this century.

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