〈Review Article〉

Molecular evolutionary medicine based on variations in hagfish lactate dehydrogenases

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Summary We considered the mechanism of human disease through genetic experiments to elucidate how hagfish lactate dehydrogenases (LDs) have adapted to the environment. The hagfish LDs acquired new properties to adapt to high water-pressure or high water-temperature during evolution. The substitutions of six amino acids (6, 10, 20, 156, 269, and 341) occurred under high water-pressure and that of one amino acid (220) occurred under high water-temperature. In human disease, mutations of four amino acids (6, 220, 314, and 320) were reported to be involved. Two amino acid sites, 6 and 220, are the same in hagfish and human and may be involved in both vertebrate evolution and human disease.

Human disease may therefore be clarified from a new viewpoint. The specific regions in DNA vulnerable to mutation may be important in molecular evolution. Changes in amino acids are discussed in relation to food abundance, human disease, and evolution.

Key words: Lactate dehydrogenase, Hagfish, Evolutionary medicine

1. Introduction

The essential tenet of the new discipline of evolutionary or Darwinian medicine is that susceptibility to malfunction and disease must in part reflect historical or evolutionary legacies[1-3]. The corollary is that we might then benefit from stepping back to take a broader look at human history and our protracted evolutionary trajectory. The evolutionary processes involved in the diversification of molecules, cells, tissues, and physiological processes rely on options generated randomly from previous templates. This is coupled with the selection of beneficial traits, by contingency or chance, or neutral drift. Evolutionary biologists continue to debate the relative importance of the mechanisms of selection, particularly as claims that traits were positively selected (the adaptionist argument) cannot always be substantiated. Irrespective of these uncertainties, the processes involved will inevitably result in "designs" that have constraints or limitations onboard and trade-off, collateral damage or negative impacts. Ultimately, inherent flaws are tolerated at some level, as long as they do not impact deleteriously on reproductive fitness. A systematic attempt to use

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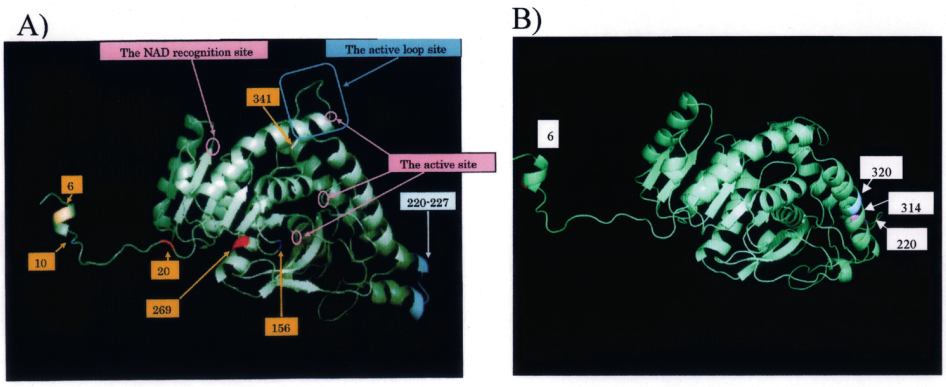

Fig. 1 Three-dimensional structures of hagfish LD-A and human LD-A.
A) Hagfish LD-A: Changes in six amino acid residues (6, 10, 20, 156, 269, and 341) in hagfish LD-A occurred with evolution. One or more of those amino acids may be involved in the development of pressure tolerance.
B) Human LD-A: Changes in four amino acid residues (6, 220, 314, and 320) in LD-A are involved in human disease.
The model of hagfish LD-A was produced by the 3D-JIGSAW modeling program (Cancer Research, UK) using the crystal structure of LD-A from dogfish, pig, and human (PDB codes 1LDM, 9LDT, and 1I10, respectively)

the concepts of evolutionary biology to elucidate human vulnerability to disease has been long in coming. An important publication in this field was the 1991 review entitled the Dawn of Darwinian Medicine by Randolph Nesse, an insightful physician, and George Williams, a distinguished evolutionary biologist[4]. In the years that followed its publication, evidence supporting the credibility of this perspective on health and illness has accumulated, and the argument was advanced that there are few areas of medical education, research, or practice that cannot be enriched by such an evolutionary view, including the scourges of obesity[5], heart disease[6], and diabetes[7]; and the enigmas of female menopause[8], aging[9], and cancer[10]. We speculate that food abundance may have caused the Cambrian explosion of species, followed by the death of Placodermi but the survival of Agnatha that were able change a key enzyme in the glycolytic pathway, lactate dehydrogenase (LD).

2. LD from humans and hagfishes

Evolutionary medicine studies diseases from the viewpoint of evolution. We propose here a new field, molecular evolutionary medicine, which focuses on genetic evolution. We are currently comparing genetic variations in relation to the development of human disease. As an example, human and hagfish LDs (EC

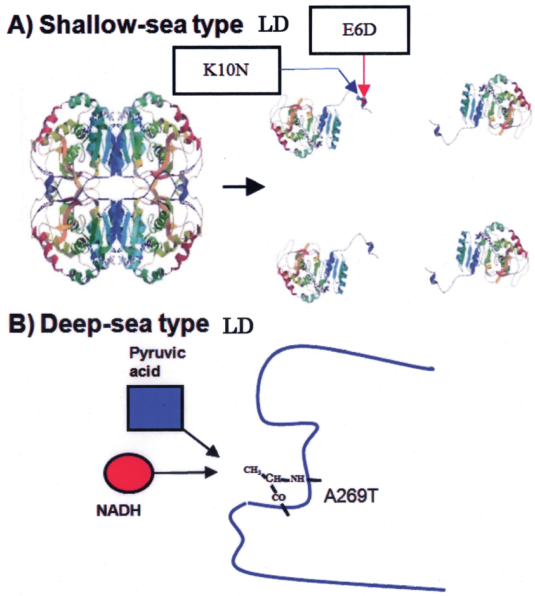

Fig. 2 Two models of pressure tolerance conferred by LD-A in hagfish.
Under high-pressure conditions, the shallow sea-type LDs dissociated from tetramers to form dimers and monomers (A), but the deep sea-type LDs did not dissociate and the structure of the substrate binding site may have been transformed (B)

1.1.1.27) are related. Six amino acids (6, 10, 20, 156, 269, and 341) of LD in hagfish were reported to be involved in the acquisition of pressure tolerance[11-16],

since differences were found in those six amino acid residues when comparing the LDs of three hagfish species living at varying depths[11]. Four of the amino acid residues (6, 10, 20, and 341) occur where four monomers combine to form tetramers, and the amino acid residues (156 and 256) neighboring the active site may control enzymatic activity (Fig. 1-A). Four amino acids (6, 220, 314, and 320) in LD were found to be involved in human disease (Fig. 1-B)[17-20]. Substitutions of amino acids 6, 220, 314, and 320 were reported to result in the fast B subunit, fast A subunit, slow A subunit, and slow B subunit variation, respectively.

Under high water-pressure conditions, the shallow sea-type LDs dissociate from tetramers to form dimers and monomers (Fig. 2A), but the deep sea-type LDs do not dissociate and consequently the structure of the substrate binding site may be transformed (Fig. 2B). The heat stability of *Eptatretus burgeri* LD-A is greater than that of *E. burgeri* LD-B. The 220-227th amino acids of *E. burgeri* LD-A, which are absent in *E. burgeri* LD-B, are therefore assumed to be involved in heat stability (as will be reported elsewhere).

Amino acids 6 and 220 of hagfish LD (tolerance sites of pressure and heat) and human LD (involved in the change from the normal to diseased state) are the same. Determining the mechanism(s) of change from the normal to diseased state may clarify the relationship between vertebrate evolution and human disease.

In the sixth amino acid of LD, that in humans changed from the normal Lys to Glu as a genetic variant. That in hagfish changed from Glu in *Eptatretus okinoseanus* to Asp in *E. burgeri* during evolution. Three amino acids (Lys, Glu, Asp) have isoelectric points of 9.74, 3.22 and 2.77, respectively. The two sixth amino acid of LD shifts for having more negative charge. In the 220th amino acid of LD, that in humans changed from the normal Lys to Glu as a genetic variant. That in hagfish is Val-His-Lys-Lys-Gly-Asp-Lys-Ser (220-227) in *E. burgeri* LD-A, but was lost over the course of evolution in LD-B. The eight amino acids (220-227) of LD-A include four basic ones. The positive-charge of residue 220 of both human and hagfish LDs was lost. Two amino acid residues, 6 and 220, acquired a charge during vertebrate evolution or through human disease. Understanding the meaning of those charge shifts

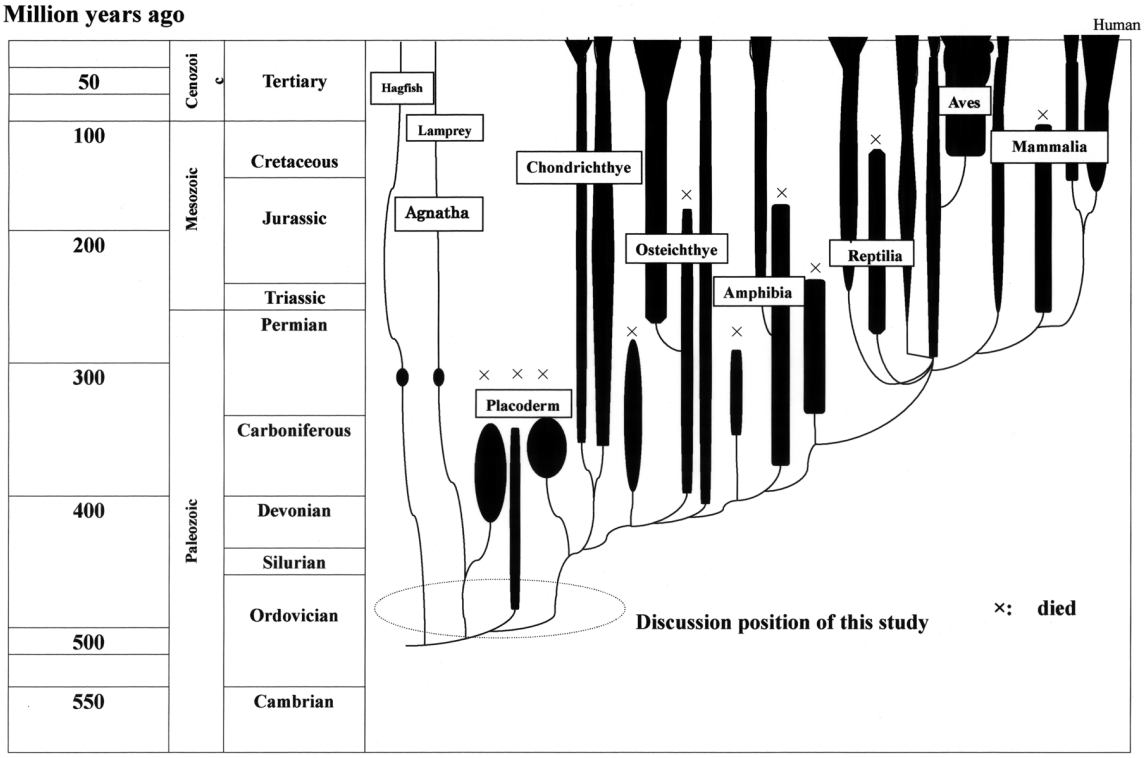

Fig. 3 Evolution of vertebrates and relative diversity of each family.

may also clarify the relationship between vertebrate evolution and human disease.

When subjected to physical pressure or high temperature, hagfish LDs exhibit new properties. Substitutions of seven amino acids (6, 10, 20, 156, 220, 269, and 341) occurred as adaptations during evolution. In human disease, mutations of four amino acids (6, 220, 314, and 320) are reported to occur. Because amino acids 6 and 220 are the same in hagfish and humans, they may be involved in both vertebrate evolution and the development of disease in humans.

3. Molecular evolutionary medicine

As evolution continued, the body structure of many species underwent two major types of variation. One type was the change from invertebrates to Agnatha and Placodermi, which occurred in the early Cambrian period (530 million years ago) when food supplies were abundant. The other type was the evolution from Osteichthye to Amphibia in the Devonian period when food supplies were scarce for many sea-dwelling species. Sea-dwellers subsequently developed lungs to replace their gills and upper and lower limbs to replace their fins (Fig. 3).

Our human ancestors who were regularly able to consume numerous types of food several times a day began to develop various diseases. Because the two phenomena of pressure tolerance and thermal stability are both governed by LD in hagfish, we extrapolate that a single hypothesis could clarify the relationships between human disease and vertebrate evolution as well as that between the food supply available at different times and evolution. Animals that consume fewer calories generally exhibit longer lifespans, as long as their nutrient intake is adequate. Therefore human disease may be seen from a new viewpoint. The regions of DNA which changed during evolution are more susceptible to further mutation than other regions, such as amino acids 6 and 220 of LD. These regions may also change when food supplies become relatively more abundant or scarcer. The evolution caused by changes in amino acids can have negative impacts, as when numerous species died out in the Devonian. As changes occur in LD as a result of human disease, the human species may also be facing a die-off in our distant evolutionary future.

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