ECOLOGICAL FACTORS AS PRESSURE OF NATURAL SELECTION OF AHR GENOTYPES IN AVIAN SPECIES

Hwang J-H[1], Park H-J[1], Bak S-M[1], Hirano M[2], Iwata H[2], Park J-Y[3], Park Y-S[1], Kim E-Y[1]

[1]Department of Life and Nanopharmaceutical Science and Department of Biology, Kyung Hee University, Seoul, Korea; [2]Center for Marine Environmental Studies (CMES), Ehime University, Matsuyama, Japan; [3]Nature Conservation Research Division, National Institute of Environmental Research, Incheon, Korea

Introduction

Dioxin-like compounds (DLCs) are one of major environmental contaminants influencing the health of various wild species. Some populations of birds in the high trophic level accumulate high concentrations of DLCs in their tissues and organs by bioaccumulation process in the ecosystem, and suffer from adverse effects such as developmental disability, immunosuppression, and teratogenesis. Aryl hydrocarbon receptor (AHR) is a key transcription regulatory factor that mediates dioxin toxicity. Despite the basic molecular mechanism of AHR-mediated signaling pathway is well conserved among vertebrates, there are large interspecies differences in sensitivity to the exposure to TCDD and other DLC compounds. The differential TCDD sensitivity in avian species has been explained by the difference in two amino acids in the ligand binding domain (LBD) of avian AHR1s. AHR1 of the chicken (*ck*AHR1) has Ile^{324} and Ser^{380} in the LBD, AHR1 of the black-footed albatross (*bfa*AHR1) has Ile^{325} and Ala^{381}, and AHR1 of the common cormorant (*cc*AHR1) has Val^{325} and Ala^{381} in the corresponding sites, showing TCDD-EC_{50} values for AHR1-mediated transactivation in the order of *ck*AHR1 (0.030 nM) < *bfa*AHR1 (0.077 nM) < *cc*AHR1 (0.36 nM) (Lee et al., 2009; Thuruthippallil et al., 2012; Thuruthippallil et al., 2013). Furthermore, evidence from *in silico* docking analyses of avian AHR1 and TCDD supported the sensitivity to TCDD in these species (Hirano et al., unpublished data). Here we hypothesize that ecological factors have been the pressure of natural selection of AHR genotypes in the evolutionary process of avian species, and eventually have delivered the interspecies difference in the sensitivity to DLCs. In the present study, we thus explored ecological factors that may have affected the selection of AHR genotypes.

Materials and methods

To understand relationship between ecological factors and avian AHR1 LBD genotypes influencing dioxin sensitivity, we collected AHR1 LBD sequences of 100 avian species deposited in GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) (Farmahin et al., 2013, Fujisawa et al., 2013). In addition to the existing data, we analyzed sequences of the AHR1 LBD amino acid residues of 14 Far East avian species collected in Korea. On the basis of these genetic data, we performed phylogenetic and statistical analyses using cDNA sequences from total 114 species.

1. Cloning and sequence analysis of AHR1 LBDs from Far East avian species

Liver and blood samples of 14 Far East avian species collected in Korea were stored in RNAlater (Qiagen, GmbH, Hilden, German). Total RNA was extracted from these samples and cDNA was synthesized from the extracted total RNA. For cDNA cloning of AHR1 LBD, a forward primer (5'-CA GAC CAA CTT CCT CCA GAG-3') and a reverse primer (5'-CGC TGC TTG CTG GAT AAC-3') were used. Amplified 640bp fragments of AHR1 LBDs were subcloned to pLUG-Prime T vector (Intron Biotechnology, Seoul, Korea) for sequencing analysis. Sequence analyses of nucleotide and deduced amino acid residues were conducted by Mac Vector 7.1.

2. Phylogenetic analysis

To understand the evolutionary process of AHR1 LBD genotypes, we initially made a phylogenic tree of 114 avian species using mitochondrial cytochrome b DNA sequences (Sibley and Ahlquist, 1990; Tamaki et al., 2013; Kimball et al., 2013) and analyzed the distribution of AHR1 LBD genotypes in the avian phylogenetic tree. We also conducted phylogenic analysis according to cDNA sequences of AHR1 LBD by PhyML (http://www.phylogeny.fr) using MUSCLE alignment and approximate likelihood-ratio test (aLRT).

3. Data collection of ecological factors and statistical analysis

We collected ecological factors of each avian species from Birds of Korea (Lee et al., 2000) and "All about birds" (http://www.allaboutbirds.org/) of the Cornell lab of Ornithology. Collected data were categorized into four ecological factors including feeding, habitat, nesting and migration types. Data were coded by presence or absence form for each ecological factor. We used PC-ORD 5.31 (MjM software design) and R (http://www.r-project.org/) to statistically analyze the link between the ecological factors and the AHR1 genotypes. The distance between data was calculated by using Euclidean algorithm, and hierarchial clustering was performed by Ward's method. We also conducted principal component analysis to explore the ecological factors that may have been the pressure of natural selection of AHR genotypes in the evolutionary process of avian species. We then operated cross tabulation test to identify the distributions of ecological factors according to AHR1 LBD genotypes by SPSS 18.0 (SPSS, Chicago, IL, U.S.A).

Results and discussion

1. Cloning and sequence analysis of Far East avian AHR1 LBDs

AHR1 LBDs of 14 avian species were obtained to add information on the sequence of AHR1 from Far East species. We succeeded in cloning avian AHR1 LBD from blood as well as liver samples. Thus this demonstrates that non-destructive blood collection made it possible to isolate the clone of AHR1 cDNA from wild species. Among examined species, AHR1 from 13 species showed a moderate sensitive type (I-A), and that from only one species, grey-headed woodpecker, showed a low sensitive type (V-A). Identities of nucleotide and amino acid sequences of AHR1 LBD among 14 species were more than 96 and 99.5%, respectively, implying that AHR1 LBDs of avian species are highly conserved.

2. Phylogenetic analysis of avian mitochondrial cytochrome b and AHR1 LBD genotypes.

To represent the evolution of avian species, we constructed a phylogenic tree based on mitochondrial cytochrome b DNA sequences of 114 avian species. The result showed that raptors, water birds, and woodpeckers have a low sensitivity type of AHR1 as a dominant type, and Passeriformes dominantly have a moderate sensitivity type. This implies that the mutation of critical amino acid residues of avian AHRs is correlated with the taxonomy or phylogeny of avian species. We also conducted phylogenetic analysis using nucleotide sequences of AHR1 LBD. The result showed that clusters were classified into the group of the same sensitive type of AHR1 LBD, suggesting the gene-specific evolution of AHR1 LBD.

3. Relationships between ecological factors and AHR1 LBD genotypes

To understand the relationships between ecological factors and AHR1 LBD genotypes, we analyzed the data by cross tabulation test and calculated the proportion of each ecological factor according to AHR1 LBD genotypes. In the case of the feeding type, birds with a high sensitive (I-S) type of AHR1 included omnivorous or herbivorous species, and birds with a moderate (I-A) type of AHR1 included species that are omnivorous, herbivorous or feed on aquatic organisms. In particular, birds with a low sensitive (V-A) type of AHR1 included all feeding types and all of the carnivorous birds belonged to this group (Fig. 1a). The proportion of migration types showed that 67% of I-S types were non-migratory birds, 71% of I-A type was migratory birds. In the case of V-A type, the proportion of the birds which occasionally migrates was higher than those in other sensitivity types (Fig. 1b). Birds with I-A type of AHR1 had the most various habitat types, and more than 50% of this group inhabits forest (Fig. 1c). In the case of V-A type, the proportion of aquatic habitat (including artificial aquatic, marine neritic, marine intertidal and marine coastal/supratidal) was higher compared to those of other sensitivity types. Regarding the nesting type, no particular association with AHR1 LBD genotype was found (Fig. 1d). On the base of the cross tabulation test, we conducted cluster analysis and principle component analysis to find the relationship between ecological factors and AHR1 genotypes. By the cluster analysis, avian species were divided in 3 clusters depending on the feeding, nesting and migration types. Cluster 1 contained the birds that feed on aquatic organisms and have a low sensitivity type of AHR1 genotype (V-A). Cluster 2 consisted of the birds that occasionally migrate, feed on small animals, and have a low sensitivity type of AHR1. Cluster 3 included migratory birds that inhabit forests, feed on buds and insects, and have a moderate sensitivity type of AHR1 (I-A). In addition, the result of principal component analysis showed that feeding, migration and habitat types explained 47.3% of the data scattering. Thus, these results suggest that certain ecological factors may have been the pressure of natural selection of AHR1 genotypes in birds.

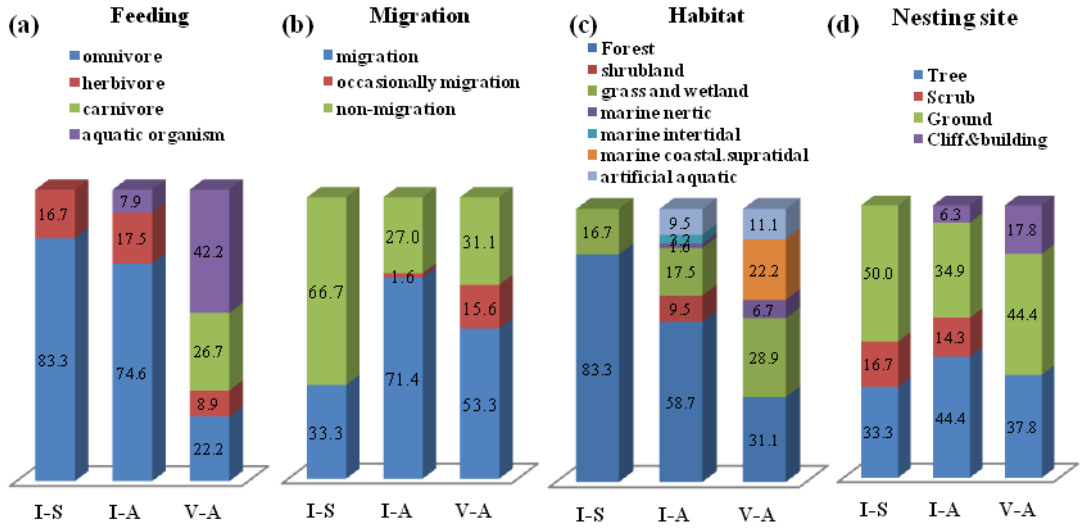

Fig 1. Results of cross tabulation test of AHR1 LBD genotypes and ecological factors. (a) Proportion of feeding types in each AHR1 genotype. (b) Proportion of migration types in each AHR1 genotype. (c) Proportion of habtat types in each AHR1 genotype. (d) Proportion of nesting site types in each AHR1 genotype.

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