Original Article

Tyrosine Hydroxylase-Immunopositive Cells and Melanin in the Mesencephalon of Yugan Black-Bone Fowl

Meng Chu[1], Yangquan Liu[1], Yu Si[1], Hang Yu[1], Yaqiong Ye[1], Haiquan Zhao[1], Hui Zhang[1,2]

[1]Department of Veterinary Medicine, College of Life Science and Engineering, Foshan University, Foshan 528231, [2]Department of Veterinary Medicine, College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang 330045, China

Abstract

Background: The Yugan blackbone fowl (YBF) is a special poultry with hyperpigmentation in various organs, including feather. However, the mechanism of hyperpigmentation is limited, and the melanic information of other organs except skin is rare. **Aims and Objectives:** In this study, we attempt to get an insight of the mechanism of melanogenesis of birds. **Materials and Methods:** The mesencephalon of YBF was observed by light microscopy with hematoxylineosin and tyrosine hydroxylase (TH) immunohistochemistry. **Results:** The TH immunopositive cells were found in the mesencephalon. Moreover, the melanin was also observed in the connective tissue of the mesencephalon. **Conclusion:** Our results confirmed the existence of melanin and TH immunopositive cells in the mesencephalon of YBF. These results provide a reference for further study on the mechanism of melanogenesis/hyperpigmentation in birds.

Keywords: Chicken, immunohistochemistry, melanin, tyrosine hydroxylase

Introduction

The black-bone fowl (BF) is a special poultry because of hyperpigmentation in various organs.[1] Other vertebrate species are also reported to show pigmentation in the viscera.[2,3] Melanin plays an important role in radioprotective, antioxidation, predator–prey interactions, thermoregulation, disease and parasites resistance, antimicrobial activity, inhibiting virus, and protecting against mitochondrial superoxide generation and mtDNA damage.[4-8] BF with hyperpigmentation may serve as a potential biological model to study the mechanism of melanogenesis and melanomagenesis.[9] Many studies regarding BF focus on the skin melanogenesis. Previous study has demonstrated the ultrastructure of melanocytes and processes of melanogenesis in the skin of BF.[1] However, the melanic information of other organs is rare. The Yugan black-bone fowl (YBF) belongs to a kind of BF and has also a great mass of melanin in many organs. In particular, their feather is flat and black. It is different from another BF – Taihe silky BF, which has white and silky feather. The Taihe silky BF was frequently studied.[10] However, the investigations of YBF are rare.

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Tyrosine hydroxylase (TH) is a rate-limiting enzyme for the biosynthesis of dopamine (DA). TH is a reliable marker for some types of neurons.[11] Further, TH is expressed in the dopaminergic cells, which are involved in melanogenesis.[12,13] Here, the TH-immunopositive cells and melanin were identified in the mesencephalon of YBF, in an attempt to understand the cytological mechanism of melanogenesis and hyperpigmentation in birds. Further, we might get insight into the pathogenesis of neuromelanin-related disease – for example, Parkinson's disease.[14]

Materials and Methods

Animals

This work had been conducted under the scientific and

Address for correspondence: Prof. Haiquan Zhao, College of Life Science and Engineering, Foshan University, Foshan 528231, China.
E-mail: fszhaohq@163.com
Dr. Hui Zhang, College of Life Science and Engineering, Foshan University, Foshan 528231, China.
E-mail: zhanghui429@hotmail.com

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technical department of Jiangxi Agricultural University after relevant ethical review. The chickens were fed *ad libitum* for 60 days, drinking-free and comfortable environment. All chickens were sacrificed with ethyl ether. After euthanized, the mesencephalons of the chickens were sampled for section and immunohistochemistry (IHC).

Mesencephalon sections and hematoxylin-eosin staining

A standard procedure in histology was used according to a previous study.[15] The samples of the mesencephalon from the chickens were fixed in Bouin's solution and then washed with 0.1 M phosphate-buffered saline (PBS) (pH 7.4), dehydrated in ethanol, and embedded in paraffin. The 5 μm sections were stained with hematoxylin-eosin for general morphology. The slides were mounted with Permount and analyzed with BM 2000 microscope (Nanjing, China). The micrographs were acquired using ScopeImage 9.0 (H3D) software (Nanjing, China).

Tyrosine hydroxylase immunohistochemistry

After deparaffinating and rehydration, the sections were heated at 95°C for epitope retrieval in citrate buffer (pH 6.0) for 15 min. The sections were treated with 0.3% H_2O_2. After washing in PBS, the sections were incubated in the normal goat serum at room temperature in a humid chamber for 30 min, and then, they were drained and further incubated in anti-TH antibody (Abcam, USA) (diluted 1:200) overnight at 4°C. The secondary antibody was applied at room temperature for 15 min, and the sections were rinsed and incubated in streptavidin-HRP at room temperature for 15 min. After washing in PBS, the peroxidase was visualized with a diaminobenzidine/hydrogen peroxidase chromogen reaction in the dark room. The sections were visualized with a BM 2000 microscopy (Yongxin, China). The color reaction was ceased by PBS rinse, and then, the sections were counterstained by hematoxylin. The photomicrographs were also acquired using ScopeImage 9.0 (H3D) software (Yongxin, China).

Results

The location of melanin in Yugan black-bone fowl

The YBF was black all over the body [Figure 1a-d], including skin [Figure 1b], muscle, bone, and viscera [Figure 1c and d]. In particular, the feather was black [Figure 1a]. The melanin was also present in the connective tissue of internal organs [Figure 1c and d]. In addition, the melanin was present in mesencephalon, cellular gray matter, and connective tissue surrounding the blood vessels [Figure 2a and b].

Tyrosine hydroxylase immunohistochemistry

IHC results showed that many TH-immunopositive cells located in the ventral mesencephalon [Figure 3a and b]. The TH-immunopositive cells were stained and showed brown granules with different shade in the cytoplasm. Some processes of TH-immunopositive cells were observed [Figure 3a and b]. The nuclei of TH-immunopositive cells and neuroglial cells were negative without brown granules. They were stained by hematoxylin and showed blue.

Figure 1: The gross anatomy of Yugan black-bone fowl. The chicken had black flat feather (a). A high amount of melanin was located in the skin (b), muscle (c), bone (c), and viscera (c and d)

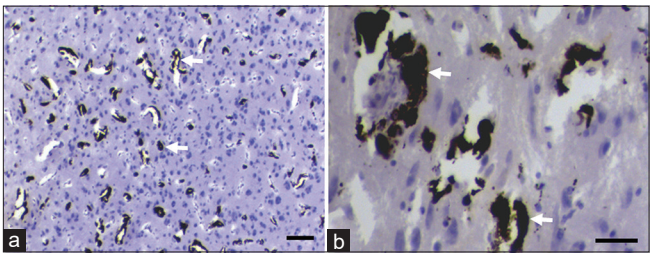

Figure 2: A large amount of melanin was located in the mesencephalon of Yugan black-bone fowl. The white arrows denoted the melanin (a and b). Bar: (a) = 50 μm and (b) = 20 μm

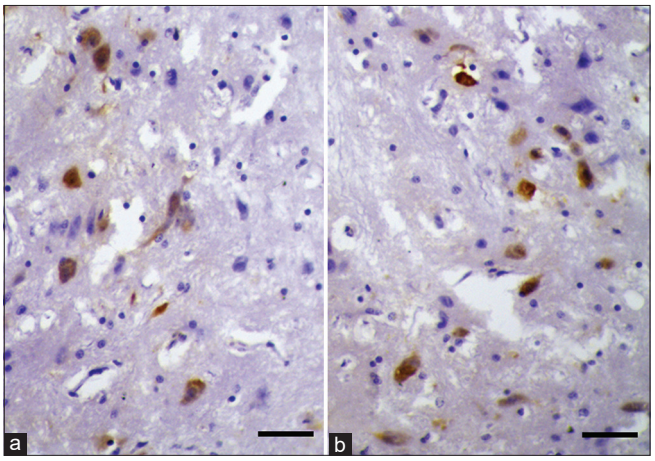

Figure 3: The tyrosine hydroxylase-immunopositive neurons were present in the mesencephalon of Yugan black-bone fowl (a and b). Bar: 20 μm

Discussion

TH is a rate-limiting enzyme of DA synthesis and is also a reliable marker for dopaminergic cells and neuronal cells.[11] TH is located in the dopaminergic cells and neuronal cells of

the peripheral system and central nervous system (CNS).[16] TH catalyzes tyrosine conversion into Dopa, which is decarboxylated to DA.[17] DA belongs to catecholamines, which also includes norepinephrine (NE) and epinephrine (E). Moreover, DA can be converted to NE and E.[17] DA can be synthesized in the CNS, but the brain is regarded as a minor source of DA.[18] However, in the present study, TH-immunopositive cells were frequently observed in the ventral mesencephalon of YBF. A previous study showed that neuromelanin has been found only in the dopaminergic neuronal cells in the human brain.[12] The neuromelanin could be synthesized from peptidyl derivatives of DA in which the Cys group of the peptide is linked to DA.[19] In addition, the retinal pigmented epithelium could be a potential source of Dopa, responsible for activating dopaminergic cells before TH synthesis.[13] In the current study, our results demonstrated that TH-immunopositive cells located in the ventral mesencephalon of YBF. It is suggested that the TH-immunopositive dopaminergic cells of the mesencephalon might produce a great deal of DA. Further studies should be done to confirm the suggestion.

Previous studies demonstrated that all catecholamines, including DA, are involved in melanogenesis and pigment aggregation in various species.[20] The catecholamines are possible precursors of neuromelanin, the biosynthesis of which occurs in brain regions containing DA and NE.[21] In particular, DA produced from TH-immunopositive dopaminergic neuronal cells could be as precursor of melanin or induce melanin synthesis directly.[21] DA also provides the cAMP response and promotes melanogenesis in melanocytes via the β_2-adrenoceptor signal.[22,23] DA can also transform into E/NE and induce melanin synthesis.[23] Therefore, TH-immunopositive dopaminergic cells may be involved in the melanogenesis by catecholamines in the mesencephalon of YBF.

Conclusion

In the present study, our results confirmed the existence of melanin and TH-immunopositive cells in the mesencephalon of YBF. It is suggested that TH-immunopositive dopaminergic cells and melanogenesis may be related by catecholamines. The results will contribute to better understand the cytological mechanism of melanogenesis and hyperpigmentation in birds.

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Conflicts for interest

There are no conflicts for interest.

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