Deficiency of 14-3-3ε and 14-3-3ζ by the Wnt1 promoter-driven Cre recombinase results in pigmentation defects

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Abstract

Background: The seven 14-3-3 protein isoforms bind to numerous proteins and are involved in a wide variety of cellular events, including the cell cycle, cell division, apoptosis and cancer. We previously found the importance of 14-3-3 proteins in neuronal migration of pyramidal neurons in the developing cortex. Here, we test the function of 14-3-3 proteins in the development of neural crest cells in vivo using mouse genetic approaches.

Results: We found that 14-3-3 proteins are important for the development of neural crest cells, in particular for the pigmentation of the fur on the ventral region of mice.

Conclusions: Our data obtained from the 14-3-3ε/14-3-3ζ/Wnt1-Cre mice strongly indicate the importance of 14-3-3 proteins in the development of melanocyte lineages.

Keywords: 14-3-3, Ywhae, Ywhaz, Knockout mouse, Cre transgenic mouse, Neural crest cell, Pigmentation, White patch, Melanocyte, Weight modulation

The repeated-epilation (Er) mutant mouse, as first reported by Hunsicker in 1960, is characterized by a loss of hair induced by radiation exposure [11]. Homozygote Er mice die at birth while heterozygotes develop normally, followed by excessive hair loss resulting in a sparse coat. Li et al. [12] showed that the repeated epilation is caused by a single nucleotide insertion in the Sfn gene, encoding the 14-3-3σ protein. In addition, they found that the skin defects seen in these mice are the result of abnormal epidermal differentiation. Thus, this indicates that 14-3-3σ proteins are important for the proper development of the epidermis.

Miller-Dieker syndrome is characterized by severe lissencephaly caused by neuronal migration defects as well as craniofacial defects [13] and is caused by a chromosomal deletion in the 17p13.3 region where the Lis1 (PAFAH1B1) and 14-3-3ε (YWHAE) genes are localized. The 14-3-3ε and Lis1 knockout mice do not show any craniofacial defects, suggesting that the 14-3-3ε protein is not important for craniofacial development. In general, 14-3-3 proteins have to form homodimers or heterodimers to function inside cells, depending on each
14-3-3 isoform. Although 14-3-3ε proteins are able to form functional homodimers, they predominantly form heterodimers with 14-3-3ζ ([14] and our unpublished observations). Therefore, we tested if 14-3-3ε and 14-3-3ζ double knockouts show any craniofacial defects resulting from defects in neural crest cell development. We achieved this by producing 14-3-3ε/14-3-3ζ double knockout mice using Wnt1-Cre transgenic mice in which Cre recombinase is expressed in neural crest cells [15–17].

Results
To analyze the functions of the 14-3-3ε and 14-3-3ζ proteins in neural crest cells, we utilized mouse genetic approaches using 14-3-3ε conditional (flox) knockout mice, 14-3-3ζ conventional knockout (KO) mice and Wnt1-Cre transgenic mice in which Cre recombinase is expressed in the neural crest cells [18]. Although the complete double knockout (14-3-3εfl/fl/14-3-3ζfl/fl/Wnt1-Cre+) mice were embryonic lethal, the 14-3-3ε+/+fl/+ζ+/Cre+ mice are able to survive to adulthood (Table 1). However, the survival rate of the 14-3-3ε+/+ζ−/+/Cre+ mice was lower than expected (Table 1, observed: n = 5, expected: n = 12), and they show decreased weight compared to the control 14-3-3ε+/+ζ+/+/Cre+ mice (Fig. 1, Control: 14.58 g ± 2.58, 14-3-3ε+/+ζ−/+/Cre+: 7.98 g ± 2.26).

The 14-3-3ε+/+ζ−/+/Cre+ mice, 14-3-3ε+/+ζ−/−/Cre+ mice, 14-3-3ε+/−ζ+/−/Cre+ mice and the 14-3-3ε+/−ζ−/−/Cre+ mice had white patches of fur on the ventral region of their torso (Fig. 2 and Table 2, 14-3-3ε+/+ζ−/+/Cre+ mice: 88.5 %, 14-3-3ε+/+ζ−/−/Cre+ mice: 80.0 %, 14-3-3ε+/−ζ+/−/Cre+ mice: 80.0 %, and 14-3-3ε+/−ζ−/−/Cre+ mice: 100 %). Interestingly, the 14-3-3ε+/−ζ+/−/Cre+ and the 14-3-3ε+/−ζ−/−/Cre+ mice did not show this phenotype. However, 14-3-3ε+/−ζ+/−/Cre+ mice did show white patches on their ventral region. The white patches were observed only on the ventral region of their torso, but not on the tail or paws or any other region. Next, we measured the area of the white patches in each genotype and summarized in Table 3 (14-3-3ε+/+ζ−/+/Cre+ mice: 0.69 cm², 14-3-3ε+/−ζ−/−/Cre+ mice: 1.09 cm², 14-3-3ε+/−ζ+/−/Cre+ mice: 0.23 cm², and 14-3-3ε+/−ζ−/−/Cre+ mice: 0.97 cm²). We found that the 14-3-3ε+/−ζ−/−/Cre+ mice, the 14-3-3ε+/−ζ−/−/Cre+ mice, and the 14-3-3ε+/−ζ−/−/Cre+ mice have larger white patches than the 14-3-3ε+/−ζ−/−/Cre+ mice. This indicates that neither 14-3-3ε or 14-3-3ζ is dominant in regulating the size of the white patches. Together, these data suggest the importance of the 14-3-3 proteins in melanocyte development.

We also analyzed the craniofacial region for defects since Cre recombinase is also expressed in the craniofacial region (Fig. 3). However, we were not able to find any pronounced defects in the craniofacial region. Further research should be performed to analyze the functions of 14-3-3 proteins in craniofacial development (see the “Discussion” section).

Discussion
We found that the 14-3-3ε/14-3-3ζ/Wnt1-Cre+ mice had white patches in their fur on the ventral region of their torso. In the Wnt1-Cre+ mice, Cre recombinase is expressed in neural crest cells which differentiate into a variety of cells, including melanocytes [18]. A previous study using Wnt1-Cre+ mice showed that the AP-2α transcription factor knockout mice had white patches similar to those seen in the 14-3-3ε/14-3-3ζ/Wnt1-Cre+ mice [19]. Neural crest cells are initially generated in the roof plate of the neural tube and migrate and differentiate into specific cells such as melanocytes. Therefore, 14-3-3 proteins could be involved in the migration and differentiation of neural crest cells. Also, it could be possible that 14-3-3 proteins are involved in melanin production in melanocytes. In addition, we cannot exclude the possibility that 14-3-3ζ is important for proper development of neural crest cells because the 14-3-3ε+/−ζ+/+/Cre+ and 14-3-3ε+/−ζ−/−/Cre+ mice showed white patches, but not the 14-3-3ε+/−ζ+/−/Cre+ (Table 2). To avoid the potential functional compensation by other 14-3-3 isoforms during embryonic development, it may be needed to analyze the functions of the 14-3-3ζ protein in pigmentation by creating and analyzing the 14-3-3ζ conditional knockout mice in conjunction with the Wnt1-Cre transgenic mice.

Table 1 Genetic ratio from mating the 14-3-3ε+/−fl/14-3-3ζ+/−/Wnt1-Cre+ mice

| Cre | − | + | − | + | − | + | − | + | − | + | − | + | − | + | − | + | − | + | − | + | − |
|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ε  | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ |
| ζ  | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ | +/− | −/+ | +/+ |
| OBS | 8  | 14 | 2  | 15 | 25 | 5  | 8  | 16 | 1  | 6  | 13 | 2  | 14 | 26 | 5  | 5  | 11 | 0  |
| EXP | 6  | 12 | 6  | 12 | 24 | 12 | 6  | 12 | 6  | 12 | 6  | 12 | 6  | 12 | 6  | 12 | 6  | 12 | 6  | 12 | 6  |

OBS observed, EXP expected

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Although there is no statistical significance in the difference in the weight between the 14-3-3ε+/ζ−/Cre− and 14-3-3ε+/ζ−/Cre+ mice (Fig. 1), the 14-3-3ε+/ζ+/ζ−/Cre+ mice tend to be smaller than the 14-3-3ε+/ζ−/ζ−/Cre− mice (Fig. 1). Obviously, the 14-3-3ζ deficiency results in the smaller body size (Fig. 1). Interestingly, the 14-3-3ε gene was removed by Wnt1 promoter-driven Cre recombinase although 14-3-3ζ was deleted in all tissues. Therefore, these data suggest that the deletion of the 14-3-3ε protein by Wnt1 promoter-driven Cre recombinases is responsible for the smaller body size in addition to the 14-3-3ζ deficiency. In addition to neural crest cells, Cre recombinase is expressed in the midbrain/hindbrain junction [16, 20]. Although food consumption was not recorded, it has previously been shown that the hypothalamus is important for controlling feeding behavior [21]. Although the expression of Cre recombinase in the hypothalamus has not been analyzed in Wnt1-Cre transgenic mice, Wnt1 mRNA is expressed in the hypothalamus, suggesting the potential expression of Cre recombinase in the hypothalamus in the Wnt1-Cre mice [22]. Also, a previous study indicated that Cre recombinase is expressed in the pituitary in Wnt1-Cre mice [23]. It is also known that the functional interaction between the hypothalamus and the pituitary is essential for their functions. Therefore, it is possible that the 14-3-3ε protein is important for the proper function of the hypothalamus and the pituitary and may alter feeding behavior and weight maintenance. To test this hypothesis, the specific ablation of 14-3-3ε in these tissues will need to be analyzed in the future.

In addition to pigmentation defects, it is possible that the ablation of 14-3-3ε and 14-3-3ζ in neural crest cells results in severe defects in other organs and tissues, such as the gastrointestinal tract and thyroid, potentially explaining the lower body weight seen in the 14-3-3ε/14-3-3ζ/Wnt1-Cre mice. The enteric nervous system in the gastrointestinal (GI) system is derived from neural crest cells [24, 25]. The enteric nervous system is required for the proper movement of food along the entire GI tract [26]. Disruption of the enteric nervous system therefore could directly impact food uptake and processing and thus interrupt normal growth and weight gain. Therefore, it is possible that the 14-3-3ε/14-3-3ζ/Wnt1-Cre mice have defects in GI peristalsis. Also, parafollicular cells, also called C cells in the thyroid, are derived from neural crest cells and secrete calcitonin involved in the regulation of calcium metabolism [27]. Parafollicular cells also secrete other small peptides such as somatostatin and serotonin, and are involved in thyroid hormone production [28, 29]. Therefore, it is possible that the 14-3-3ε/14-3-3ζ/Wnt1-Cre mice have defects in controlling hormone production in the hypothalamic-pituitary-thyroid axis due to a dysfunction in these cells or in their localization in the thyroid, which is essential for the regulation of metabolism [28, 29]. Thus, further research should be performed to investigate these potential defects by measuring the concentration of the hormones, such as thyroid stimulating hormone (TSH), T3 and T4, in the 14-3-3ε/14-3-3ζ/Wnt1-Cre mice.

Regarding the craniofacial development in the 14-3-3ε/14-3-3ζ/Wnt1-Cre mice, we were not able to find any significant defects in the craniofacial region. However, more research on this topic needs to be undertaken before reaching a conclusion. Further experiments including histology and immunohistochemistry such as bone staining by Alcian Blue/Alizarin Red should be done. Also, it is very helpful for better understanding the mechanisms of craniofacial development to use other Cre transgenic mice, including earlier developmental Cre expression than the Wnt1 promoter can provide, and compare the results obtained from the analyses using these different Cre transgenic mice. The P0 (protein 0)-Cre transgenic mouse is another frequently used mouse line in which Cre recombinase is expressed in epithelial layers of developing tooth germ and taste buds [30]. Also, another Cre transgenic line, tamoxifen-inducible Sox10-Cre transgenic mice in which the expression of Cre recombinases can be regulated by the administration of tamoxifen, will be useful to analyze potential defects in greater detail [31, 32]. Thus, a combinatorial use of a different Cre transgenic mouse lines will provide knowledge for understanding the precise mechanisms of craniofacial development.
Conclusions

Analysis of the functions of 14-3-3ε and 14-3-3ζ proteins indicates their importance in the development of neural crest cells, in particular the development of the melanocyte lineage. Also, our data suggest that the 14-3-3ε proteins are important for weight modulation during development.

Fig. 2  14-3-3 ablation in neural crest cells caused the formation of white patches on the ventral region. Photos were obtained at P21. Note that the 14-3-3ε+/ζ+/+/Cre + mice do not have white patches, but the 14-3-3ε+/ζ+/+/Cre mice have white patches. Arrows in upper panel mark white patches.
Methods

Mice

The 14-3-3ε conditional (flox) mice and the 14-3-3ζ conventional knockout (KO) mice are described previously [2, 4]. The 14-3-3ε flox mice and the 14-3-3ζ KO mice have been maintained in the 129 genetic background by continuing to backcross them with 129SVE inbred strain.
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