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論 文 要 旨

学 位 論 文
要 約

表 題 バゾプレッシンV1a受容体欠損マウスにおける加齢変化促進の機序解明と応用 (Analysis and application of aging pigment accumulation in adrenal gland of v1a vasopressin receptor knockout mouse)

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表題

バゾプレシン V1a 受容体欠損マウスにおける加齢変化促進の機序解明と応用

Analysis and application of aging pigment accumulation in adrenal gland of V1a vasopressin receptor knockout mouse

1 研究目的

Lipofuscin is an autofluorescent pigment that accumulates in aging cells. The first description of this lipofuscin in the literature was by Hueck in 1912 and Hannover in 1842. In addition to a value for a convenient marker of aging, lipofuscin is often involved in age-related diseases. It has been known that the adrenal cortex of rodents and humans accumulates lipofuscin with age. How this aging process proceeds is not known. We previously found that lipofuscin deposition was accelerated in the adrenal cortex of mice lacking V1a vasopressin receptor gene (V_{1a}KO). In the adrenal cortex, arginine vasopressin (AVP) exerts two main effects by acting on the V_{1a} receptor subtype: it increases the mitogenic activity of the zona glomerulosa and stimulates aldosterone and glucocorticoid secretion in mice, rats, and humans. However, besides these hormonal functions of AVP, it remains unknown whether AVP plays a role in the production and accumulation of aging pigments. This study was conducted to clarify the time course of lipofuscin accumulation in the adrenal cortex of V1aKO mice. We also identified accompanying changes in the gene expression profiles by comparing the adrenal cortices of V1aKO and wild-type (WT) mice.

2 研究方法

Experiment I

The aim of this experiments was to measure the distribution of lipofuscin in the adrenal cortices of V_{1a}KO and WT mice. We utilized two groups of mice of both sexes at age of 2, 6, and 12 months. To quantitate the amount and timing of lipofuscin deposits, we have collected 60 adrenal glands. Rhodamine phalloidine and DAPI were applied to membrane and nucleus staining, respectively. The slides were examined under confocal microscope and digital images were recorded. The area and localization of lipofuscin in adrenal slices were examined by Image-J software.

Experiment II

Gene expression profile was analyzed by using cDNA microarray in the adrenal cortex of male mice at 2 months age. The experiments were performed as previously described and according to the manufacturer's instructions. The expression of selected genes was confirmed by quantitative RT-PCR. The total RNA was isolated from adrenal gland (n = 10) and cDNA was synthesized by SuperScript III first strand synthesis reagent kit. Activity of MMP-14 (membrane-type 1 matrix metalloproteinase) in the adrenal cortex were analyzed according to the manufacturer's instructions (AnaSpec Inc., CA, USA).

Experiment III

To investigate adrenal V_{1a} -mediated cellular signaling, we analyzed mouse Y1 adrenocortical cell line. The cells at a density of 5×10^5 cells/35-mm glass base dish were treated with buffer (control) or 50nM AVP (treatment) for 0, 3, 6, and 12 hours. The total RNA was isolated from Y1 cells and cDNA was synthesized. The expression of target genes was measured by qRT-PCR. RT-PCR was performed to exam the expression levels of V_{1a} , V_{1b} or oxytocin receptors in Y1 cell.

3 研究成果

Experiment I

At 2 months of age, lipofuscin autofluorescence was detected in the adrenal cortex of V_{1a} KO male mice, but not of WT mice. High-magnification images showed that lipofuscin accumulation began in the cytoplasm and augmented up to the age of 12 months. Importantly, significantly larger deposits were detected in V_{1a} KO mice. These findings suggest that V_{1a} KO mice showed accelerated aging changes that were observed in the normal aging process.

Experiment II

From the expression profiles, we found two up-regulated genes, *Xaf1* and *Serpina1b*, and down-regulated genes, *Adi1* and *Abhd1*, in the adrenal cortex of V_{1a} KO. Our qRT-PCR analysis performed on new adrenal samples confirmed the results of microarray experiments. The MMP14 activity was significantly higher in the adrenals of V_{1a} KO mice than those of WT mice. Together with the result of reduced *Adi1* expression level in the KO adrenals, it can be speculated that *Adi1* regulates cellular morphology and migration of adrenocortical cells in V_{1a} KO through the activation of the MMP14.

Experiment III

PCR analysis revealed that V_{1a} subtype was the main vasopressin/oxytocin receptor in Y1 cells. In fact, application of AVP (10^{-7} M) stimulated a prompt increase in intracellular Ca^{2+} concentrations [Ca^{2+}]_i in Y1 cells. This [Ca^{2+}]_i was suppressed by the V_{1a} antagonist SR49059. Furthermore, when V_{1a} receptor was stimulated by AVP, *Xaf1* expression in Y1 cells was significantly suppressed. These results indicate that the AVP and V_{1a} receptor system plays an important role in regulating the expression of aging-related genes.

4 考察

Experiment I

V_{1a} KO lipofuscin can be detected as early as at 2 months of age. Accumulation of lipofuscin was augmented in both of mice groups especially in 12 months, but larger deposits were detected in the V_{1a} KO mice group. Notably, the calculated area of lipofuscin fluorescence in the adrenal sections of male V_{1a} KO increased 6.8 times from 2 to 12 months of the age. Unexpectedly, this number in the WT adrenal sections was the similar level of 6.7 times. These results suggested that the effect of V_{1a} deletion already started in the 2 months of age and continued throughout our observation period.

Experiment II

The transcripts of differentially expressed four genes were significantly altered between the adrenal cortices of WT and V_{1a} KO mice. *Xaf1* and *serpina1b* expression in V_{1a} KO mice were increased than WT mice. *Xaf1* increases p53 and plays an important role in cell fate between apoptosis and survival. *Serpina1b* is one of five α_1 -protease inhibitor proteins known to regulate neutrophil elastase. In contrast, *Adi1* encodes a multi-functional protein with significant homology to bacterial acireductone dioxygenase and regulates mRNA processing in the nuclei. *Adi1* is also known as the membrane-type 1 matrix metalloproteinase MMP-14-binding protein. We examined the possibility of altered enzymatic activity of

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MMP14 in adrenal cortex of V1aKO mice. The exact role of Abhd1 is not known. However, a family protein, Abhd5, is involved in lipid metabolism in the liver. Among these four genes, we focused on Xaf1, because this gene was reported to interact with the p53 tumor suppressor protein and enhance the non-tumor related functions of p53, such as senescence.

Experiment III

RT-PCR analysis revealed that V_{1a} is a main vasopressin receptor in Y1 cells. The application of AVP (10^{-7} M) stimulated prompt increase in intracellular Ca²⁺ concentrations [Ca²⁺] in Y1 cell, which was suppressed by V_{1a} antagonist. Finally, when V_{1a} was activated by AVP, Xaf1 gene expression in Y1 cells was significantly suppressed. These results indicated that V_{1a} and AVP system plays an important role in regulating transcripts for aging-related genes.

5 結論

In summary, this study delineated an enhanced aging phenotype in the adrenal cortex of V1aKO mice by examining the time course of lipofuscin accumulation. The V1a receptor in the adrenal cortex significantly impacts the gene regulatory network. The four genes selected in this study are likely candidates for the enhanced aging phenotype in V1aKO adrenal glands. Responsible gene(s) for adrenal aging should be identified in further studies.