NISR Research GRANT
2007 Research Grant

Basic and applied studies on functional factors in fermented foods

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Research aims

Prevention of life style-related diseases with the use of food functions is highly required for the purpose of a reduction of healthcare cost in an aging society, Japan. Most life style-related diseases are triggered by obesity occurring in association with disruption of lipid metabolism homeostasis. It is known that a variety of nuclear receptors are involved in the diseases. Forty-eight human nuclear receptors are activated by inherent ligands bound to their C-terminal domain, but some receptors are still referred to as orphan receptors due to no ligands identified. Retinoic acid receptor-related orphan receptor α (RORα) is one of orphan receptors, biological functions of which remain uncertain, though cholesterol sulfate and its derivatives are speculated to be endogenous specific ligands (1). We found that RORα is expressed in adipocytes and that the activities of its unidentified endogenous ligand elevate during adipogenesis. In this study we investigated biological functions of RORα in adipocytes on the basis of the assumption that fermented foods would contain a wide variety of steroid derivatives mimicking functions of RORα ligands.

Methods

Mouse 3T3-L1 preadipocyte cells were differentiated into adipocytes as previously described. A reporter plasmid containing a GAL4 DNA-binding domain followed by the RORα ligand-binding domain (LBD) was constructed to evaluate the ligand-binding activity. The amounts of mRNA in adipocytes were determined by the real-time PCR method. 3T3-L1 cells were infected with a lentivirus expression vector for either RORα or shRNA of RORα, and were differentiated.

Results

Expression of RORα elevates during adipocyte differentiation. 3T3-L1 cells were differentiated into adipocytes and total RNA was recovered. The mRNA level of aP2, one of differentiation markers, increased after day 4 after differentiation, and the mRNA level of RORα also similarly did up to 5 times. Western blot analysis with specific antibodies against RORα revealed that the protein level also elevated. These results suggest that RORα regulates the expression of its target genes during adipogenesis.

The RORα ligand activity was found in the culture medium of adipocytes. Because adipocytes accumulate lipid droplets in their cytosol during adipogenesis, it is postulated that the content of lipid soluble ligands of nuclear receptors would increase. The expression of PPARγ, a master regulator of adipogenesis, elevates in the early stage of differentiation in association with the increase in its endogenous ligand, thereby being activated. It has been reported that the unidentified PPARγ ligand is secreted in the culture medium of adipocytes (2). Luciferase assays were carried out using the RORα reporter gene together with the PPARγ reporter gene as a positive control. When the cells transfected with one of the reporter genes were incubated with a culture medium for day 6 differentiated adipocytes, the RORα ligand activity was detected in the culture medium as well as the PPARγ ligand activity. We failed to find any activities for some other nuclear receptors tested in the assays. Taken together, it is suggested that the increased RORα during adipogenesis in association with the increased particular ligands would regulate its target gene expression in adipocytes.

Overexpression of RORα restrains adipogenesis. RORα was overexpressed about 10-fold in 3T3-L1 cells with the lentivirus expression vector, then the cells were differentiated. The overexpression decreased lipid droplets in adipocytes and down-regulated the expression of differentiation-related genes. In particular, the expression of perilipin, a protein located on the surface of lipid droplets and essential for lipid droplet formation, was reduced by RORα. As a result, it is likely that the lipid droplet formation was repressed by the function of RORα (3).

RORα knowkdown stimulates adipogenesis. The endogenous RORα level in 3T3-L1 cells was reduced down to less than 30% with the RORα shRNA expression lentivirus vector. When the cells were differentiated, more lipid droplets were formed and the expression of differentiation-related genes was induced. These results opposed to those observed in the RORα overexpressing cells clearly indicate that endogenous RORα has an inhibitory effect on adipogenesis.

Conclusion

It is thought that PPARγ, a master regulator of adipogenesis, stimulates differentiation of adipocytes through the

increased its expression in the early and middle stage of differentiation, and the elevated amount of its ligand. RORα intensively studied in the current report behaved quite similarly to PPARγ during adipogenesis, and was speculated to play a pivotal role in adipocytes. Indeed, its overexpression depressed adipogenesis, while its knockdown stimulated it. The fact that the expression of perilipin, a lipid droplet surface protein essential for its formation, is particularly downregulated by RORα raises the possibility that RORα would have a kind of anti-obesity function. Although we tried to construct a reliable assay system to evaluate the RORα ligand activity, the assay using some fermented food factors was unable to be performed due to no potent synthetic ligand as a positive control. The RORα ligand activity found in the culture medium of adipocytes in this report was only 2- to 3 - fold compared to that in the control medium. The more sensitive assay system should be established by the use of endogenous or synthetic potent ligand that will be found in the near future.

References

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