Depletion of the Arylhydrocarbon Receptor (AhR) during Adipose Differentiation in 3T3-L1 Cells

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Introduction

The arylhydrocarbon receptor (AhR) is the receptor for 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) and the related compounds. Upon binding to the ligand, the AhR translocates to nucleus followed by the formation of heterodimer with the AhR nuclear translocator (Arnt). This heterodimer binds to specific enhancer sequence (termed xenobiotic response element; XRE) within the promoter region of various genes (1-3).

TCDD, a highly toxic compound that has recently attracted much attention as an environmental contaminant, is primarily deposited into adipose tissue in vivo. In adipose tissue, the inhibitions of glucose transport, lipoprotein lipase activity and fatty acid synthesis are reported as a result of the presence of TCDD (4-7). These inhibitory effects of TCDD in adipocytes may be the results of the interaction with AhR. However, there is little information concerning of states of the AhR during the adipose differentiation.

In this study, we examined the level of the AhR during the adipose differentiation in 3T3-L1 cells.

Materials & Method

3T3-L1 cells were maintained and differentiated as described elsewhere (8). Proteins were detected with the specific antibodies against the AhR and the Arnt, and its expressions were quantitated with the use of National Institutes of Health Image 1.61 soft. DNA binding activities in the nuclear extracts were determined by electrophoretic mobility shift assay using the double-strands

oligonucleotides containing XRE. To judge the statues of adipose differentiation by visual inspection, cultures were fixed with 10% formalin and stained with 0.5% Oil red O solution. Glycerophosphate dehydrogenase activity was assayed by monitoring the decrease in absorbance at 340 nm of NADH in the presence of dihydroxyacetone phosphate (9).

Results & Discussion

The level of the AhR protein was found to decrease with ongoing adipose differentiation in 3T3-L1 cells. The binding activity to xenobiotic response element and the cellular response to TCDD were also lowered as the result of the adipose differentiation. These results indicate that the depletion of the AhR is the novel event associated with adipose differentiation in 3T3-L1 cells and that the magnitude of the depletion of the AhR is of sufficient to lose the functional response to xenobiotics in 3T3-L1 cells.

We found the population of 3T3-L1 cells which have the capability of adipose differentiation even in the presence of high dose of TCDD. These cells lack the nuclear AhR but not the cytoplasmic AhR, suggesting the possible negative role of the liganded nuclear AhR in adipose differentiation.

The level of the Arnt protein was also decreased as a result of the differentiation. However, the pattern of the depletion of the Arnt protein was distinct from that of the AhR protein.

The data presented in this study will provide opportunities to carry out studies to better understand the roles of the AhR in adipose cells where is the primary target of TCDD.

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