ARYLHYDROCARBON RECEPTOR (AHR) IS INVOLVED IN NEGATIVE REGULATION OF ADIPOSE DIFFERENTIATION IN 3T3-L1 CELLS

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Introduction

The arylhydrocarbon receptor (AhR) is a ligand-activated transcription factor that mediates a spectrum of toxic and biological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and the related compounds. The AhR is postulated to play important roles not only in the regulation of xenobiotic metabolism but also in the maintenance of homeostatic functions. In cell differentiation, the AhR is increased during differentiation toward keratinocytes and monocytes[1,2]. We previously reported that AhR protein is found to decrease with ongoing adipose differentiation in 3T3-L1 cells, resulting in the loss of functional response to xenobiotics[3]. The studies using TCDD-resistant clone of 3T3-L1 cells suggested that AhR may be involved in the negative regulation of adipogenesis. To confirm this hypothesis, in this study, 3T3-L1 cells were stably transfected with a full-length mouse AhR subcloned into expression vector in either orientation of sense or antisense, and the potency of adipose differentiation in these cells was examined.

Methods and Materials

3T3-L1 cells were maintained and differentiated as described elsewhere[3]. Full length of murine AhR cDNA was subcloned into the mammalian expression vector pRc/CMV2 (Invitrogen), which contain a selective marker, the neomycin resistance gene. The cells were transfected by using Lipofection (Life Technologies, Inc.) and were allowed to grow in nonselective medium for 48h. The cells were then cultured in medium containing G-418. After 2 to 3 weeks, clones were isolated and expanded individually. The expression of the AhR was analyzed by western blotting. To judge the states of adipose differentiation by visual inspection, cultures were stained with 0.5% Oil red O. Total RNA was extracted using TRIzol reagent (Life Technologies, Inc.) from the cells according to the manufacture's instruction and analyzed by Northern blotting. Probes correspond to C/EBPs, PPAR, aP2 and CYP1B1 were prepared by RT-PCR. Cell cycle analysis was carried out with FACScan (Becton Dickinson).

Results & Discussion

To directly assess the participation of AhR in adipogenesis, we, first, established cell clones from 3T3-L1 cells by stable transfection with vector expressing high level of AhR sense mRNA or antisense of AhR mRNA. Comparison of the differentiation potency of these clones to control cells showed that overexpression of the AhR suppressed morphological differentiation as well as induction of adipocyte-related genes, while the lowering of the AhR induced much greater morphological differentiation and expression of adipocyte-related genes in cells. Activation of PPAR _ with the ligand can promote adipose differentiation. Therefore,

we next examined whether the activation of PPAR __can rescue the diferentiation potential in the sense cells. In the sense cells, conventional differentiation cocktail or ligand alone had no effect on adipogenesis. However, combination of the differentiation cocktail and PPAR _ ligand allowed the sense cell to accumulate lipid droplets as much as in the control cells. We also examined the effects of the AhR on clonal expansion in adipogenesis. In a first set of experiments, wild type 3T3-L1 cells were treated with 10nM TCDD and the cell proliferation was determined. Treatment of the cells with TCDD suppressed the cell growth in 3T3-L1 cells. Similarly, overexpression of AhR slowered the cell proliferation, whereas the lowering of the AhR protein stimulated the cell growth. Cell cycle analysis revealed that, after 18h of stimulation with differentiation-medium, most of sense cells were remained in G0/G1 phase, whereas half of the control cells are entered in S phase. In antisense cells, somewhat enhanced entry into S-phase was observed relative to that seen in the vector cells.

Consequently, we are led to conclude that the AhR appears to play a negative regulatory role in adipose differentiation process in 3T3-L1 cells in the absence of exogenous ligand.

References

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