INTRODUCTION

PPARγ is a key transcriptional regulator of adipogenesis, and is thought to be involved in the development of high fat diet-induced obesity. Our previous studies have revealed that PPARγ and its ligands promote adipogenic differentiation and suppress chondrogenic differentiation in growth plate chondrocytes (1). Leptin is a cytokine-like hormone secreted predominantly by adipocytes. Lack of leptin protein causes obesity in mice. However, the growth plates of leptin-deficient ob/ob mice show disturbed columnar structure, decreased type X collagen, increased apoptosis and premature mineralization (2), suggesting a possible role of leptin in regulating the function of the growth plate. The purpose of this study therefore was to investigate the regulatory role of leptin compared to PPARγ-activation in growth plate chondrocytes.

METHODS

Chondrocytes were isolated from the resting zone of the distal femoral growth plate of 2-day old rats. Cells were resuspended in DMEM/F12 medium supplemented with ITS+1 and plated in monolayer at a density of 5×10^5 cells/cm², or in a pellet culture of 1×10^5 cells/ml. Cells were treated with leptin and ciglitazone at concentrations of 1 μg/ml and 5 μM, respectively, or infected with PPARγ adenovirus (kindly provided by Dr. J. L. Jameson) at an MOI of 100. Total RNA was isolated from cells infected with Ad-PPARγ or treated with ciglitazone for 7 days in the presence or absence of leptin. Real-time PCR reactions were conducted to evaluate the gene expression of the markers of chondrogenic differentiation (Col2a1, Sox9), terminal differentiation (Col10a1) and adipogenic differentiation (aP2). The cell proliferation rate was assessed using MTT based cell growth determination kit from Sigma. Apoptosis of chondrocytes was assessed using Apo-ONE Caspase-3/7 Assay Kit from Promega. Protein levels of anti-apoptotic Bcl-2 and pro-apoptotic Bax were examined by immunoblotting.

RESULTS

Laser capture microdissection followed by quantitative real-time PCR revealed that both leptin and the leptin receptor were expressed in growth plate chondrocytes. Treatment of chondrocytes with the PPARγ ligand ciglitazone inhibited the expression of leptin mRNA (Figure 1). Leptin partly reversed the inhibition of Col2a1 and Sox9 mRNA expression induced by ciglitazone (Figures 2A and 2B). MTT assay showed that ciglitazone reduced the chondrocyte cell proliferation rate compared to controls, and this reduction was attenuated by addition of leptin (Figure 2C). Ciglitazone-induced inhibition of T3-mediated terminal differentiation was also partially reversed by leptin (Figure 2D). Caspase 3/7 activity was increased in ciglitazone-treated or Ad-PPARγ-infected cells, and these increases were also reduced by addition of leptin (Figure 2E). Immunoblotting demonstrated that leptin decreased Ad-PPARγ-induced Bax expression, and also attenuated the reduction in Bcl-2 expression triggered by Ad-PPARγ infection. Finally, ciglitazone-induced expression of the adipocyte marker aP2 was only mildly suppressed by leptin (Figure 2F).

DISCUSSION

We confirm in this study that activation of PPARγ inhibits chondrogenic differentiation, proliferation and terminal differentiation, and promotes apoptosis in growth plate chondrocytes. These effects may result from PPARγ-mediated reduction of leptin levels in the growth plate. Exogenous leptin partially reverses the inhibitory effects of PPARγ on the chondrogenic differentiation and proliferation of growth plate chondrocytes, as well as the adipogenic changes induced by PPARγ. This relative lack of leptin in the growth plate might contribute to the mechanisms that lead to dysfunction of the growth plate that occurs in children with the obesity-related diseases, such as slipped capital femoral epiphysis and adolescent Blount’s disease.

REFERENCES