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PPARgamma (Peroxisome proliferatoractivated receptor gamma) interacts with Sp1 transcription factor on the p21waf1/cip1 promoter region to stimulate its transcription in cladosporol A – treated HT-29 cells

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Dietary habits and life-style are the major etiological factors of colorectal cancer and, therefore, the use of chemopreventive agents able to interfere with the disease onset is widely accepted. In a previous work, we demonstrated that cladosporol A, a secondary metabolite purified from *Cladosporium tenuissimum*, is able to inhibit the growth of colon cancer cells through the upregulation of p21waf1/cip1 gene expression mediated by an Sp1-dependent mechanism. In different cell types the increased p21waf1/cip1 transcription has been shown to depend on a specific Sp1/PPARgamma interaction. In this work we sought to investigate the functional relationship between these transcription factors in HT-29 cells treated with cladosporol A.
PPARgamma protein levels undergo a cyclic reduction due to a negative autoregulation mechanism triggered by cladosporol A, supporting the notion that it is a PPARgamma specific ligand.
This result is confirmed by the reciprocal effect observed after addition of GW9662, a well-recognized irreversible antagonist.
The concomitant exposure of HT-29 cells to cladosporol A and GW9662, indeed, attenuates the increase of p21waf1/cip1 protein levels further proving the involvement of PPARgamma in the upregulation of p21waf1/cip1 gene expression.
Transient silencing of PPARgamma in HT-29 cells exposed to cladosporol A reduces the upregulation of the endogenous p21waf1/cip1 gene and inhibits the transcriptional activation of an exogenous transiently transfected p21waf1/cip1 promoter. Coimmunoprecipitation assays show that PPARgamma specifically interacts with Sp1, either in untreated and cladosporol A-treated cells. Finally, ChIP assays demonstrate the presence of a stronger PPARgamma/Sp1 complex on the p21waf1/cip1 promoter region along with other markers of transcriptional activation. To our knowledge, this is the first report describing cladosporol A as a novel PPARgamma natural ligand.