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Glucocorticoid Rescues Bmal1-induced Apoptotic Cell Death

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Dexamethasone (Dex), a potent analog of glucocorticoid is known as a humoral synchronizer of the mammalian circadian clock in the peripheral tissues. In this study, we examined the effect of Dex on subcellular localization of enhanced green fluorescence protein-tagged Bmal1 (Bmal1-EGFP) and Bmal1-induced apoptotic cell death. Cytoplasmic translocation of nuclear Bmal1-EGFP was not observed in HeLa cells after Dex treatment, but nuclear body formation was observed when Dex was treated Bmal1-EGFP -expressing HeLa cells. Apoptotic cell death was induced by Bmal1-EGFP expression in HeLa cells. Cleavage of poly(ADP-ribose) polymerase and DNA fragmentation were evident in BMAL1 expressed HeLa cells. Dex treatment significantly blocked cell death induced by Bmal1-EGFP. Northern blot analysis revealed that expression of anti-apoptotic Bcl-2 was up-regulated by Dex treatment only when Bmal1-EGFP was expressed in HeLa cells. Bmal1 was co-localized glucocorticoid receptor (GR). To further investigate interactions between glucocorticoid receptor and BMAL1, we examined the co-immunoprecipitation assay. Bmal1 was co-immunoprecipitated with GR and BRG1, a co-activator of GR-mediated transcription. These results suggest a possible role of BMAL1 in cell death.

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Differential Action of Raloxifene on the Expression of Estrogen Receptor α, β and the Truncated Estrogen Receptor Product-1 (TERP-1) in the Hypothalamus and Pituitary of Rat

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Recently, a truncated estrogen receptor product-1 (TERP-1) has been identified as an isoform of the rat ER that contains only the C-terminal region of ER. TERP-1 does not affect transcription directly but suppresses ligand-activated ER actions via forming heterodimer with ER. Thus, TERP-1 appears to be an endogenous ER-antagonist and may contribute to the organ-specific actions of endogenous or synthetic estrogens which are called a selective estrogen receptor modulators (SERMs). It is therefore temping to speculate, that SERMs may affect expression of ER and TERP in a different manner. To elucidate this hypothesis, we compared the effects of raloxifen (R) and estradiol (E) on the expression of ER, and TERP-1 in the pituitary and hypothalamus of ovariectomized (ovx) rats. Both compounds were applied orally twice a day. After 3 days of treatment rats were decapitated then brains and pituitaries were collected. As expected, E suppresses LH levels while R caused a slight increase compared to the values in ovx controls. While expression of ERin the pituitary was not affected by neither E or R, mRNA levels of ER increased about twofold in E-treated ovx rats. A propounded effect was observed for expression of TERP-1 mRNA levels in R treated rats and its values were 12 fold higher than those in the control. In the POA, E reduced TERP-1 mRNA levels while R caused a slight increase of expression. In conclusion, expression of TERP-1 is differentially regulated by R and E. It appears that at least part of the organ selective effect of SERM is mediated through modulation of TERP-1.

E127

Pre-mRNA Splicing of the Mouse GnRH: Interactions of 9G8 and SRp30c, Two SR Proteins

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The alternative splicing of the first intron (intron A) of mouse gonadotropin-releasing hormone (GnRH) pre-mRNA requires a downstream exonic splicing enhancer (ESE). It appears that ESE could function cooperatively to increase the efficiency of intron A removal. For instance, Tra2 was proved to specifically interact with ESE4 on the GnRH exon 4. In the present study, a panel of recombinant baculovirus -expressed SR proteins (Tra2, 9G8, SRp30c, SRp55, SRp20 and SRp40) was produced and tested for the ability to activate ESE-dependent GnRH pre-mRNA splicing *in vitro*. Among tested SR proteins, 9G8 dramatically increased GnRH intron A excision. In addition, SRp30c, which is known to interact with Tra2, could strongly bind to the ESE3 and exon 4 domain of GnRH pre-mRNA. However, SRp30c could not bind to the ESE4, suggesting the presence of additional *cis*-element on GnRH exon 4. Although SRp30c and Tra2 alone could not transactivate the GnRH pre-mRNA splicing in the absence of other nuclear components, overexpression of Tra2, 9G8 and SRp30c in NIH3T3 cells, significantly increased GnRH intron A splicing activity *in vivo*. Moreover, co-transfection of SRp30c with 9G8 or Tra2 additively augmented the excision of GnRH intron A. Taken together, these results indicate that Tra2, 9G8 and SRp30c may play an important role in the tissue-specific excision of GnRH intron A in a cooperative manner.

E128

Regulation of Human *Period*1 Promoter Is Involved with CREB Activation by MAP Kinase Pathway in Human Neuroblastoma Cell Line

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In mammals, the suprachiasmatic nuclei (SCN) of the hypothalamus function as the major biological clock. SCN-dependent rhythms of physiology and behavior are regulated by changes in the environmental light cycle. Recent work has revealed that photic stimulation during the night triggers rapid activation of *mPer1* via the mitogen activated protein kinase (MAPK) pathway in the SCN. These observations led us to examine the role of the MAPK pathway in *hper1* promoter. Here we report that CRE-binding protein (CREB) acts as a major effector of converging via MAPK signaling pathways to the *hPer1* promoter. *hPer1* promoter contains cAMP-reponsive element (CRE) and E-boxes. The *hPer1*promoter is responsive to synergistic activation of the PKA and MAPK pathway, and induction of *hPer1*promoter that requires integrity of the CRE. Therefore in the absence of the CRE, transient disruption of MAPK signaling blocks the synergistic activation of the *hPer1* promoter. In contrast, activation of *hPer1*promoter by CLOCK/BMAL1 occurs regardless of an intact CRE. Taken together these results indicate that CREB acts as a endpoint of signaling pathways for the regulation of *hPer1* promoter.