Title	The induction of p21/WAF1/CIP1 expression by the specific kappa opioid receptor agonist is independent of MAPK and PKC
Author(s)	Poon, J; Wong, N
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together, these results indicated that the cytotoxic effects of KMJ-60 (S) were associated with the induction of apoptotic cell death and G2/M arrest through regulation of several major growth regulatory gene products, and this novel topoisomerase inhibitor warrants a further study toward a potential chemotherapeutic drug.

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Expression pattern of the AP-1 family in rat adrenal infused with ACTH and FGF2

R. F. Baccaro, C. F. Lotfi; Anatomy, Biomedical Science Institute, São Paulo, Brazil

Nuclear proto-oncogenes of the Fos and Jun families are components of the AP-1 (activating protein-1) transcription factor. AP-1 is induced by hormones and growth factors signals that are implicated in the proliferation, differentiation and transformation of cells. As different dimer combinations recognize different sequence elements in the promoters, AP-1 activity can be regulated by dimer composition. In order to obtain further information on the trophic mechanism of action of ACTH (adrenocorticotropin) and FGF2 (fibroblast growth factor) in an architectural-preserved intact adrenal cortex gland, we used an *in situ* isolated rat adrenal infusion method to study the expression pattern and role of the AP-1 family transcription factors. We analyzed c-Fos, Fra-1, Fra-2, FosB and c-Jun, JunB, JunD protein expression in rat adrenal gland infused with 10^{-7}M ACTH or/and 20ng/ml FGF2. Sprague-Dawley male rats were anesthetized and the left adrenal gland was isolated and the abdominal aorta cannulated. The gland was provided with DMEM/heparin (30min) and 2h treated or not treated using an infusion pump (150-200µl/min). The glands were processed and the protein expression was determined by immunohistochemistry. In glomerulosa zone ACTH increased Fra-1 expression but had not effect in Fra-2, FosB or c-Jun protein expression, whereas FGF2 increased Fra-2 expression but decreased FosB and c-Jun protein expression. In fasciculata and reticularis zones ACTH increased c-Jun expression but FGF2 caused marked reduced expression of this protein. ACTH and FGF2 combination markedly reduced Fra-1 and c-Jun expression in all zones analyzed. Our results indicate that ACTH and FGF2 are capable of differentially regulate Fos and Jun protein families in this experimental model. AP-1 family of transcription factors is probably implicated in the regulatory cell cycle mechanism of ACTH in adrenal gland. Support by FAPESP and CAPES.

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Transcriptional Control of HiNF-P, a Critical Cyclin E/CDK2/NPAT Responsive Cell Cycle Regulatory Factor that Activates Histone H4 Gene Expression at the Onset of S Phase

R. Xie, P. Mitra, J. L. Stein, A. J. van Wijnen, G. S. Stein; Department of Cell Biology and Cancer Center, University of Massachusetts Medical School, Worcester, MA

Histone nuclear factor P (HiNF-P) is a recently discovered 65 kDa Zn finger transcription factor that activates histone H4 gene transcription at the G1/S phase transition. HiNF-P is the ultimate link of an E2F independent signaling pathway that is responsive to the cell cycle dependent stimulation of NPAT by cyclin E and CDK2. HiNF-P is expressed in multiple proliferating cell types and detectable at very early stages of mammalian embryogenesis. To define the gene regulatory pathways that control the proliferation-related expression of HiNF-P, we have now characterized the regulation of the mouse HiNF-P locus. Using transient reporter gene assays with 5' and intronic genomic segments, we show that the HiNF-P locus contains a single 0.6 kB promoter which suffices for transcriptional control. The HiNF-P promoter is phylogenetically conserved among mammalian species, is GC-rich, contains many CpG doublets and lacks a TATA-box. Promoter deletion analysis shows that there are three distinct activating regions (-400/-200, -200/-100 and -100/-1) that contribute to maximal promoter activity and contain putative recognition motifs for multiple factors. We systematically examined the contribution of candidate transcription factors to HiNF-P gene regulation using co-transfection experiments with expression vectors and promoter deletion/reporter constructs. We find that HiNF-P gene transcription is activated by SP1 in multiple cell types through a minimal promoter (-100/-1). Furthermore, HiNF-P promoter activity can be modulated by distal elements that integrate cell cycle regulatory signals to suppress (i.e., E2F1 interaction with -200/-100) or activate (i.e., NPAT/HiNF-P interaction with -400/-200) HiNF-P gene transcription. We conclude that HiNF-P gene transcription is controlled by ubiquitous gene regulators and an NPAT-related auto-regulatory mechanism to ensure fidelity of HiNF-P gene expression during the cell cycle and development.

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Density-dependent changes in intracellular redox status in IEC-6 cells

M. S. Attene-Ramos, K. Kitiphongspattana, K. Ishii-Schrade, H. R. Gaskins; University of Illinois, Urbana, IL

Intracellular reduction-oxidation (redox) potential has been implicated in the regulation of a variety of cellular processes, including cell proliferation and differentiation. However, the magnitude, direction and temporal relationship of redox changes to cellular responses are incompletely defined. The present work sought to characterize redox and metabolic changes associated with proliferative stages through contact inhibition in rat intestinal epithelial cells (IEC-6). Concentrations of redox couples [glutathione/glutathione disulfide (GSH/GSSG); NAD(P)H/NAD(P)$^+$], reactive oxygen species (ROS), and the ATP/ADP ratio were measured using HPLC or flow cytometry in IEC-6 cultures over a 12 day period. Reduction potentials were calculated using the Nernst equation; $\Delta E = \Delta E°- (61.5\ mV/n)\ \ln([Red]/[Ox]),(pH=7.2)$ with the GSH/GSSG and NAD(P)H/NAD(P)$^+$couples. From the first day of culture until one day prior to confluence (day 3), an increase ($P < 0.05$) in GSH concentrations and a significant reduction in the redox potential of the GSH/GSSG couple (35 mV) were observed. These changes were accompanied by a decrease in ROS and relative NO concentrations and an oxidation in the redox potential of the NADPH/NADP$^+$ couple (> 40 mV). Post-confluent cells exhibited a significant decrease in GSH concentrations and a significant oxidation of the GSH/GSSG couple (45 mV). The decreased GSH concentrations likely reflect the decreased ($P < 0.05$) protein expression of γ-glutamylcysteine synthetase (γ-GCS; immunoblot), the rate limiting enzyme in GSH synthesis. After day 6, relative ROS concentrations increased significantly while that of NO remained unchanged. The ATP/ADP and NADH/NAD$^+$ ratios did not change significantly (days 1-12). Together these data provide new insight into changes of the major intracellular redox couples during normal cell proliferation and contact inhibition, and set a numerical framework for future research of the mechanisms governed by intracellular redox status.

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The induction of p21/WAF1/CIP1 expression by the specific kappa opioid receptor agonist is independent of MAPK and PKC

J. Poon, N. Wong; Biochemistry, The University of Hong Kong, Hong Kong, Hong Kong Special Administrative Region of China

Abstract In a previous study, we demonstrated that apoptosis induced by staurosporine could be potentiated by the kappa opioid receptor agonist, U50,488H, suggesting that this agonist is able to enhance cellular stress. The signaling mechanism by which U50488H induce stress in an epithelial cell line is investigated in the present study. Incubation of the human epithelial cancer cell line CNE2 with U50488H resulted in the arrest of cells at the Go/G1 phase and a gradual decrease in cell number. Additionally, the expression of the cyclin-dependent kinase inhibitor p21/WAF1/CIP1 is upregulated, as demonstrated by Western Blot analysis. The co-incubation of cells with U50488H together with a general opioid antagonist (naloxone), or a specific kappa-opioid antagonist (nor-BNI) was unable to prevent the increase in p21/WAF1/CIP1 expression. The inhibition of intracellular MEK activity with PD98059, or PKC with Go6983, had no effect on the expression of p21/WAF1/CIP1 in response to U50488H, as detected by Western Blot as well as immunostaining. The chelation of intracellular calcium ion with BAPTA, or the blockade of the Inositol-1, 4,5-trisphosphate receptor with 2-APB, also did not prevent U50488H-induction of p21/WAF1/CIP1. U50488H up-regulated the mRNA-level of p21/WAF1/CIP1, but the activity of a luciferase reporter driven by the p21/WAF1/CIP1 promoter containing the Sp1/2/3, p53, C/EBPα, RAR sites was not increased. Our data suggest that U50488H stimulated the expression of p21/WAF1/CIP1 through a kappa-opioid receptor independent mechanism that does not involve the MEK or the PKC signaling pathways.

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The Tyrosine Phosphatase SHP-1 Interacts with Cyclin-Dependent Kinase-2 Protein in Epithelial Cells

J. Boulanger, C. Duchesne, M. Simoneau, N. Rivard; Anatomy and Cell Biology, University of Sherbrooke, Sherbrooke, PQ, Canada

Protein-tyrosine phosphatase SHP-1 is expressed at high levels in hematopoietic cells and at moderate levels in many other cell types including epithelial cells. While SHP-1 has been shown to be a negative regulator of multiple signaling pathways in hematopoietic cells, very little is known however as to the biological role and partners of SHP-1 in epithelial cells. To learn about the role of SHP-1 in intestinal epithelial cells, we used the yeast two-hybrid system for identifying proteins that bind to the SHP-1 protein. **Methods:** An expression vector was constructed by fusing the GAL4 DNA-binding domain to SHP-1 protein. This bait plasmid was co-transformed in yeast with a prey plasmid containing a human intestinal epithelial cDNA expression library fused to the GAL4 activation domain. To confirm the specificity of the interaction, co-immunoprecipitation experiments were performed in the epithelial cell lines Caco-2/15 and HEK293. **Results:** 1- 1/12 clones were positive in the screening and all of them contained a 1100 bp insert whose sequence was identical to a part of cyclin-dependent kinase-2 (Cdk2) cDNA sequence. 2- The interaction between SHP-1 and Cdk2 observed in yeast was confirmed by crossed co-immunoprecipitations in HEK293 overexpressing SHP-1 and Cdk2 proteins and in Caco-2/15 cells. 3- Ectopic expression of SHP-1 in HEK293 cells had no effect on endogenous Cdk2 kinase activity. 4- By contrast, ectopic expression of Cdk2 (but not dominant-negative form of Cdk2) in HEK293 cells enhanced SHP-1 phosphatase activity by 4-fold. 5- Finally, co-precipitated Cdk2 efficiently phosphorylates SHP-1 protein, as evaluated in a kinase assay. **Conclusion:** Taken together, our results indicate that SHP-1 is one of the main binding partners and a substrate for Cdk2 in human epithelial cells. Therefore, Cdk2 may be involved in the regulation of SHP-1 function.