has been initially performed using a non-radioactive fluorescently labeled oligonucleotide probe in traditional gel-shift assays, with ongoing work toward using these fluorescent probes in solution binding assays, so that binding conditions may be more closely controlled.

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125.7

Transforming Growth Factor-beta1 Regulation of Collagenase-3 Expression in Osteoblastic Cells by a Cross-Talk between Smad and MAPK Signaling Pathways and their Components, Smad2 and Runx2

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TGF-β plays a key role in osteoblast differentiation and bone development and remodeling. Collagenase-3 (matrix metalloproteinase-13) is expressed by osteoblasts and seems to be involved in osteoclastic bone resorption. Here we show that TGF-β1 stimulates collagenase-3 expression in the rat osteoblastic cell line, UMR 106-01 and requires *de novo* protein synthesis. Dominant negative Smad2/3 constructs identified that Smad signaling is essential for TGF-β1-stimulated collagenase-3 promoter activity. Inhibitors of the ERK1/2 and p38 MAPK pathways, but not the JNK pathway, reduced TGF-β1-stimulated collagenase-3 expression, indicating that p38 MAPK and ERK1/2 pathways are also required for TGF-β1-stimulated collagenase-3 expression in UMR 106-01 cells. These inhibitors did not prevent nuclear localization of Smad proteins but they inhibited Smad-mediated transcriptional activation. We have shown for the first time that Runx2 (a bone transcription factor), a potential substrate for the MAPK pathway is phosphorylated in response to TGF-β1 treatment in osteoblastic cells. Co-transfection of Smad2 and Runx2 constructs had co-operative effect on TGF-β1-stimulated collagenase-3 promoter activity in these cells. We further identified ligand independent physical interaction between Smad2 and Runx2. Taken together, our results provide an important role for a cross-talk between Smad and MAPK pathways and their components in expression of collagenase-3 following TGF-β1 treatment in UMR 106-01 cells.

125.8

Cytotoxicity, p53, RARE, Cyclin-D1, and c-fos Expression in Human Leukemia (HL-60) Cells Exposed to Arsenic Trioxide

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Arsenic trioxide has recently been successfully used to treat all-trans retinoic acid (ATRA) resistant relapsing acute promyelocytic leukemia. However, its cytotoxic and molecular mechanisms of action are poorly understood. In the present study, we used human leukemia (HL-60) cells as a model to study the cellular and molecular mechanisms of anti-cancer properties of arsenic trioxide. We hypothesized that arsenic-induced expression of stress genes and related proteins may play a role in the cellular and molecular events leading to toxicity and destruction of leukemia cells. To test this hypothesis, we performed the MTT-assay for cell viability, and Western Blot analysis to assess the expression of specific cellular response proteins including p53, RARE, Cyclin-D1, and c-fos. Densitometric analysis was performed to determine the relative abundance of these proteins. Data obtained from the MTT assay indicated a strong dose-response relationship with regard to the therapeutic properties of arsenic trioxide. Upon 24 hours of exposure, the chemical dose required to cause 50% reduction in cancer cell viability (LD50) was computed to be 6.35  0 .57 μg/mL. Western Blot and densitometric analyses also demonstrated a strong dose-response relationship with regard to p53, and RARE expression within the dose range of 0-10 μg/mL. Expression of c-fos was up-regulated within the dose range of 0-5 μg/ mL, and down-regulated within the dose-range of 5-10 μg/mL, probably due to cell death at higher level of exposure. No statistically significant differences ($p>0.05$) in Cyclin-D1 expression was found between controls and treated cells.Taken together, these results indicate that arsenic trioxide is highly cytotoxic to human leukemia cells, and is able to cause oxidative stress and cell cycle arrest through activation of the c-fos transcription factor, and the 53-kDa tumor suppressor protein. Up-regulation of RARE by As2O3 indicates that its toxicity may be mediated through interaction/binding with the retinoic acid receptor, and subsequent inhibition of growth and differentiation.

Key words: Arsenic trioxide, HL-60 cells, cytotoxicity,cyclin-D1,c-fos,p53,RARE

125.9

Increase in Ambient Temperature Enhances Gene Transfer into Human Smooth Muscle Cells

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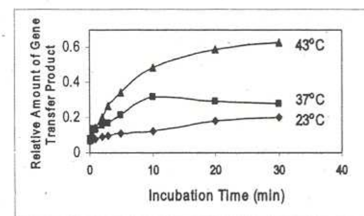
Background: Several techniques are currently used to transfer genes into various cells, tissues and organs. Although gene therapy is a potential therapeutic approach for arterial restenosis and angiogenesis, the efficiency of transfection is relatively low.

Methods: Human aorta smooth muscle cells were cultured in F12K medium. Swine growth hormone gene transfection was performed by calcium phosphate coprecipitation at various temperatures: 23°C, 37°C and 43°C. Swine growth hormone expression was detected using an indirect ELISA.

Results: The heated cultured human aorta smooth muscle cells had a significantly higher expression of the transfected growth hormone gene. Ambient temperature rise to 43°C for 30 min provided greater transient transfection of the swine growth hormone gene when compared to ambient temperatures at 37°C and 23°C ($p<0.01$). The greatest effects occurred within 10 min of incubation and persisted up to 30 min.

Conclusion: The results suggest that even a few degrees of ambient temperature rise can significantly increase gene transfer into smooth muscle cells. This may be of value when using gene therapy with transfection procedures.



125.10

Collagen alpha 1(I) gene (COL1A1) regulation by RFX family of proteins

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Type 1 collagen, the most abundant member in the collagen family, consists of two alpha1(I) (COL1A1) chains and one alpha2(I) chain (COL1A2). Previously, we demonstrated that regulatory factor for X-box (RFX) family of proteins bind at the start site (-1 to +20) of COL1A2. It is well documented that both COL1A1 and COL1A2 genes are coordinately expressed in fibroblasts. Therefore, we investigated COL1A1 gene regulation by RFX family of proteins. RFX1 does not bind to the homologous sequence of COL1A1 gene as judged by gel shift assay using fibroblast nuclear extract. However, RFX1 can interact with an upstream sequence (-11 to +6) especially when it is methylated. The COL1A1 RFX1 binding sequence in COL1A1 contains 3 CpG sites that are methylated in some cancer cell lines which poorly express the COL1A1 gene. RFX5, a family member that is not methylation sensitive but is induced by interferon gamma, also binds to the COL1A1 gene. The RFX5 complex, containing RFX5, RFXAP and RFXB proteins along with the class II transactivator (CIITA), binds with similar binding affinity to both collagen type I genes. RFX1, RFX5 complex and CIITA repress COL1A1 transcription in transient co-transfection assays. RFX1 represses COL1A1 promoter activity less than COL1A2 promoter activity, whereas RFX5 represses COL1A1 promoter activity more than COL1A2 promoter activity. Thus, the RFX