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INVOLVMENT OF p21(WAF1/CIP1) IN MITOMYCIN C-INDUCED G2 ARREST IN LP1-1 CELLS

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It has been well known that p21(WAF1/CIP1) as a cell cycle regulator inhibits an activation of cyclin-dependent kinases(CDKs) thereby blocking the G1/S transition. However, its function at G2/M checkpoint is still unclear. Therefore, we investigated whether p21 is involved in G2 phase in mouse fibroblast LP1-1 cells. After treatment with mitomycin C, DNA damaging agent, at early G2 phase, the cells were arrested at G2 phase. In addition, the activation of M-phase promoting factor(MPF) which is responsible for the M phase initiation was decreased to 50% of untreated group at late G2 phase. To investigate if p21 has a role in G2 arrest, we first obtained 351 nucleotides of p21 cDNA from NIH 3T3 cells using a RT-PCR. Using the p21 clone as a probe, mRNA levels of it were measured during the cell cycle. The mRNA levels of p21 were high until entry into S phase, showed low levels at mid S phase, increased to maximal levels at G2 phase and then rapidly decreased thereafter. In mitomycin C-treated groups, the mRNA levels of p21 at G2 phase were slightly increased compared with those of control. These results suggest that p21 is involved in mitomycin C-induced G2 arrest in LP1-1 cells and this arrest may be controlled by increasing mRNA level of p21.

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CERAMIDE-MEDIATED SIGNAL TRANSDUCTION AND c-jun GENE EXPRESSION DURING DIFFERENTIATION OF U-937 CELLS.

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Effects of ceramide on c-jun gene expression during differentiation of U-937 cells and the mechanism of ceramide-activated protein phosphatase(CAPP) pathway were studied. Ceramide induced c-jun gene expression in a time- and dose-dependent manner. The half-life of c-jun mRNA was 30 min. In contrast, inhibition of protein synthesis with cycloheximide in the absence of transcription with actinomycin D increased the half-life of c-jun mRNA in ceramide-treated U-937 cells to greater than 90 minutes. In order to investigate whether ceramide-induced c-jun gene expression is regulated through ceramide-mediated pathway, ceramide and okadaic acid were treated to the cells. Okadaic acid inhibited enhancement of c-jun mRNA induced by C_2-ceramide in dose-dependent manner. Also, in order to elucidate whether PKC-and PKA-signaling pathway are involved in c-jun gene expression by ceramide-mediated pathway, PMA and db-cAMP were added to U-937 cells exposed to okadaic acid. PMA and db-cAMP-treated group showed increased level of c-jun mRNA, but these increase could not be blocked by the addition of okadaic acid. These results demonstrated that ceramide induced c-jun gene expression during differentiation in U-937 cells and it is regulated posttranscriptionally. In addition, we suggest that the regulation of c-jun gene expression in response to ceramide might be involved the activation of ceramide-mediated pathway and this ceramide-mediated pathway is distinct from the PKA- and PKC-pathway in c-jun gene expression.