P095 Involvement of p21 in cross talk between p65 and p53 in doxorubicin-induced cell death

Lu Yan Jun, Tang Juan Juan: *Cancer Res. Center, Celstar Bio-Pharm. Co. Ltd.*

P53 acts to inhibit cancer cell growth and also to mediate cancer cell death. NFkB /p65 is a transcription factor that can protect or contribute to cell death. Here we show that knockdown of p65 by IκBSR or p65 small interfering RNA remarkably decreased the cytotoxic effect of DOX on HCT116 ($p53^{+/+}$) cells, correlating with increased induction of p21 by p53. We further demonstrate that p65 limited p53-induced p21 expression by competition with endogenous transcription coactivators p300/CBP. In previous work, we demonstrated that p21 suppressed cell death via its CDK-binding and CDK-inhibitory activity. Thus, we propose that the p65 activity is required for p53-induced cell death through limitation of p53-induced p21 expression after treatment with DOX. In HCT116 ($p53^{-/-}$) cells, downregulation of p65 expression enhanced the cytotoxic effect of DOX, due to decreased p21 expression levels. We present evidence that in p53-null tumor cells, p65 is involved in induction of p21 expression by directly binding to the p21 promoter. These findings indicate an association between p21 expression and resistance to cell death through p65 activation, a novel regulatory mechanism in which p21 bridges a transcriptional crosstalk between p53 and p65.

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Lu Yan Jun, Tang Juan Juan: *Cancer Res. Center, Celstar Bio-Pharm. Co. Ltd.*

P096 The time-dependent changes in *p21* and *bax* gene expression in epithelial cells of glandular stomach and colon in mice treated with MNU or MNNG orally

Emiko Yamauchi, Yohei F ujiishi, Kayoko Komatsu, Wakako Ohyama, Nobuyoshi Yasutake, Kazumi Uchida, Masaharu Onoue: *Yakult Central Institute for Microbiological Research*

We are trying to develop an *in vivo* test that evaluates the genotoxicity on gastrointestinal tract utilizing the gene expression changes. DNA microarray is a useful tool to select genes which expression is changed by mutagens. At first, to determine the appropriate time for microarry analysis, we examined the time-dependent changes in expression of *p21* and *bax*, which is expected to respond to mutagens. Male BALB/c mice were orally given a single dose (100mg/kg) of *N*-nitroso-*N*-methylurea (MNU) or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG). The epithelial cells of glandular stomach and colon, and liver were collected at 0.5, 2 and 4 hours post treatment. The total RNA was extracted using Tri-zol and the quantitative RT-PCR analysis was performed. The *p21* expression was significantly increased in stomach (approximately 2-fold) and liver (5- to 89- fold) in mice treated with MNU or MNNG compared with those treated with vehicle after 2 and/or 4 hours. In colon, no increase was observed. It was considered that MNU and MNNG were inactivated soon and not reached to colon. There were no changes in *bax* expression in three tissues. From the result of *p21* expression changes, we consider that it is suitable to search responding genes at 2 or 4 hours after MNU or MNNG treatment by microarray analysis.

マウス消化管上皮細胞における、変異原物質経口投与後の*p21*および*bax*遺伝子の発現変動
山内恵美子、藤石洋平、小松加代子、大山ワカ子、安武信義、内田和美、尾上正治：ヤクルト中央研究所