PARP-1 induced cytoplasmic signaling pathways

PD 105589

Poly(ADP-ribose) polymerase (PARP-1) regulates different kinase pathways but the mechanistic model for the regulation of a nuclear enzyme of the predominantly cytoplasmaticly regulated kinase pathways has yet to be identified in many cases. We raised the possibility that PARP-1 inhibition activates MAPK phosphatases-1 (MKP-1) and inactivates JNK and p38 MAP kinases in oxidative stress. These data indicated the significance of PARP-1 regulated MKP-1 expression, but the precise mechanism has not been determined.

Because regulatory region of MKP-1 has heat shock responsive element and a cAMP response element (CRE), we hypothesized that these transcription factors may play role in MKP-1 expression under oxidative stress induced PARP-1 activation. Our aim was to investigate the effects of HSFs and CREBs under oxidative stress induced PARP-1 activation. Our results show that suppression of HSF and CREB1 do not have any effect but the suppression of ATF4/CREB2 significantly decreased the MKP-1 expression.

PARP-1 induced poly(ADP-ribosyl)ation plays an important role in regulation of transcription factors through ADP-ribosylation of its partner proteins or by physical association with transcription factors. Our aim was also to investigate the precise mechanism of PARP-1 on MKP-1 expression. Our results show that PARP-1 activation in oxidative stress induced poly-ADP-ribosylation of ATF4 and inactivated its binding to CRE giving a mechanistic way to the inactivation of ATF4 dependent MKP-1 expression. PARP inhibitor dependent protection against ROS induced mitochondrial depolarization and cell death can be regulated by PARP-1-ATF4-MKP-1-JNK-p38 MAPK dependent retrograde pathway. These data indicate the identification of

a new PARP mediated retrograde pathway by which PARP inhibition through ATF4 activation activates MKP-1 expression which inactivates JNK and p38MAPK, and protects mitochondrial integrity and prevents cell death.

The effect of hydrogen peroxide on the expression of MKP-1 in HSF-1,2,4 silencing WRL-68 cells

WRL-68 liver cells transfected with a plasmid expressing of HSF-1,2,4 siRNA were treated with 0.3 mM H_2O_2 for 3 hrs. Cellular expression of MKP-1 was expression assessed by immunoblotting using a specific primary antibody. Our result shows that level of MKP-1 was not decreased in HSF-1,2,4 suppressed cells (Fig.1 A-C).

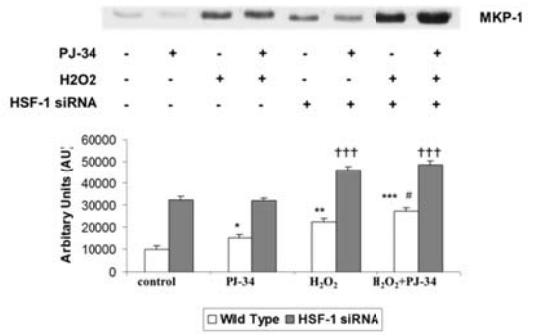

Fig.1.A

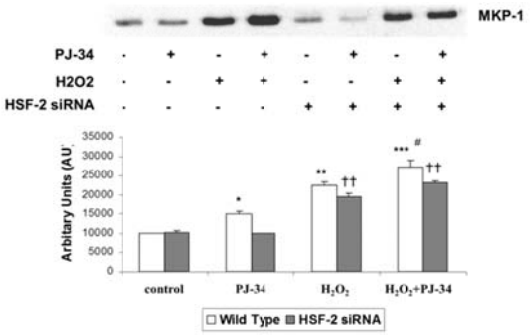

Fig.1.B

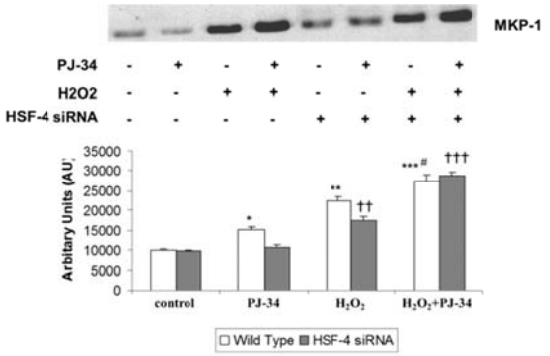

Fig.1.C

The effect of hydrogen peroxide on the expression of MKP-1 in ATF4 silencing WRL-68 cells

WRL-68 liver cells transfected with a plasmid expressing of ATF4 siRNA were treated with 0.3 mM H_2O_2 for 3 hrs. The H_2O_2-treatment significantly increased the phosphorylation of JNK1/2 and p38 MAP kinase; however, the PJ-34 with the H_2O_2-co-treatment only slightly decreased the phosphorylation of these kinases. Phosphorylation of JNK1/2 and p38 MAP kinases show the same result in the ATF4 suppressed cells, but the level of the stress induced MAP kinases were significantly increased compared with the wild type cells (Fig.2.A,B). Cellular expression of MKP-1 was expression assessed by immunoblotting using a specific primary antibody. Our result shows that level of MKP-1 was significantly decreased in ATF4 suppressed cells (Fig.2.C). These results suggest that ATF4 is able to regulate MKP-1 activation.

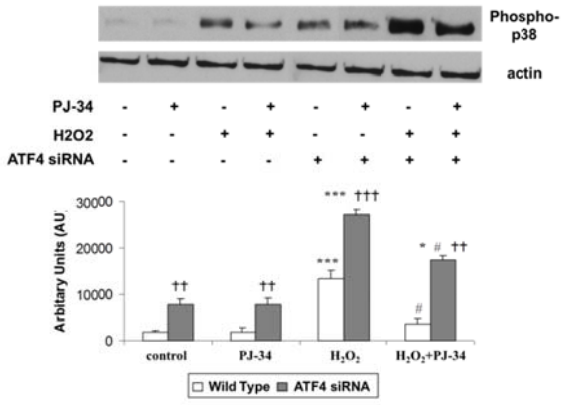

Fig.2.A

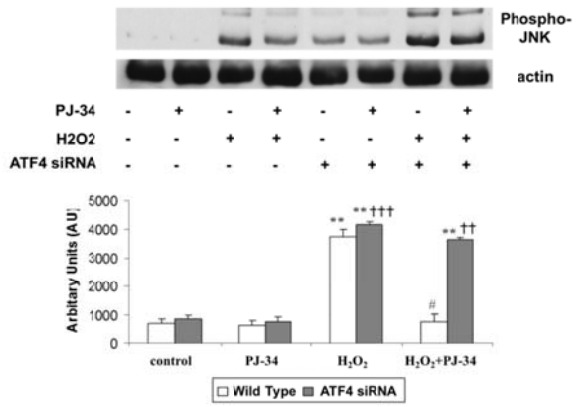

Fig.2.B

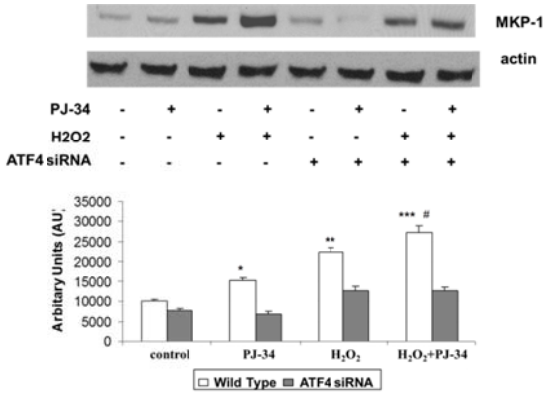

Fig.2.C

Effects of MKP-1, ATF4 suppression and oxidative stress induced mitochondrial depolarization

Since mitochondrial depolarization can be the consequence as well as the cause of both apoptotic and necrotic cell death, we studied mitochondrial membrane potential of control, 0.3 mM H_2O_2 with or without 10μM PJ-34 for 30 min in wild type and MKP-1, ATF4 suppressed cells by fluorescence microscopy. Inhibition of PARP-1 protected the cells against oxidative stress, as demonstrated by an increased fluorescence value of JC-1 red and a decreased mitochondrial depolarization induced JC-1 green fluorescence value. Although, these protective effect of PARP-1 inhibition was abolished in MKP-1 and ATF4 suppressed cells (Fig.3.A).

Statistical analysis of mitochondrial depolarization was verified with flow cytometric measurement. Result shows that oxidative stress induced significant mitochondrial depolarization in wild-type, MKP-1 and ATF-4 suppressed cells. PARP-1 inhibition was able to protect the mitochondria against depolarization in wild-type cells, although this protective effect was abolished in MKP-1 and ATF4 suppressed cells (Fig.3.B-D).

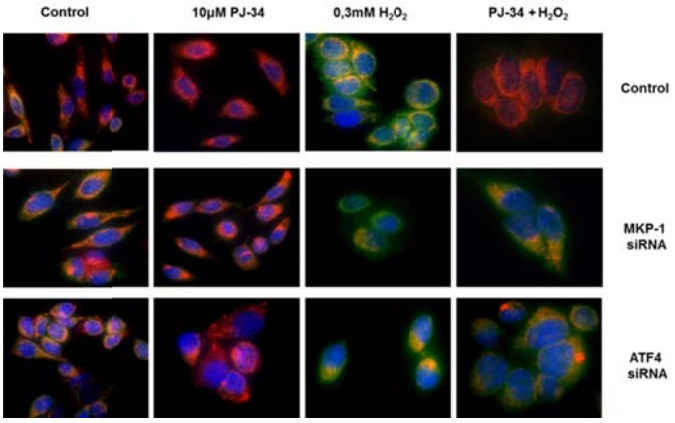

Fig.3.A

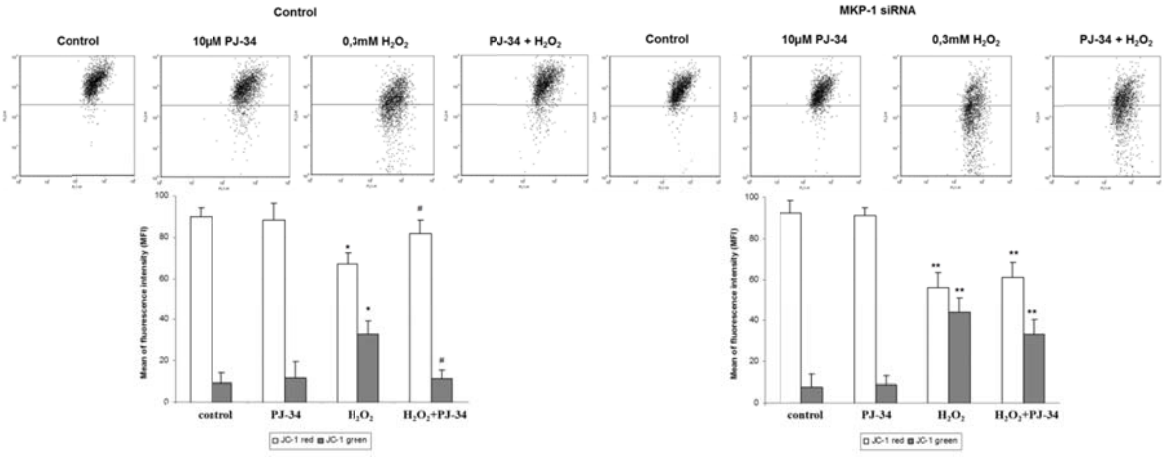

Fig.3.B

Fig.3.C

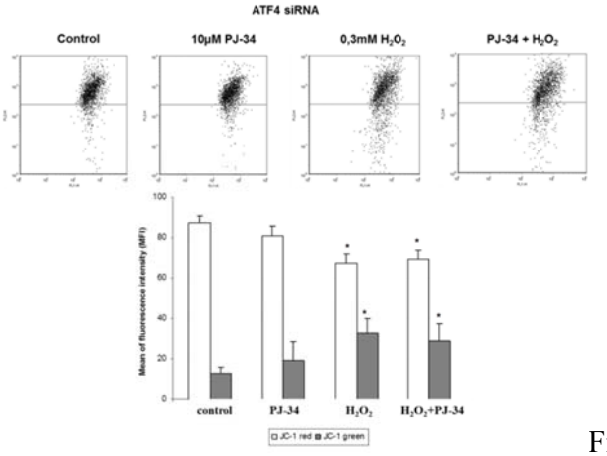

Fig.3.D

ADP-ribosylation of ATF4

Since the increased ADP-ribosylation by PARP-1 can trigger the cell death pathways and it able to modify the regulation of transcription factors, we studied poly(ADP-ribosy)lation of control, 0.3 mM H_2O_2 with or without 10μM PJ-34 for 30 min in WRL-68 cells. PAR level was increased in oxidative stress treated cells, while PARP-1 inhibition was effective by PJ-34, it decreased the PAR levels.

To test if ATF4 is a substrate by ADP-ribosylation under oxidative stress condition, cells were treated with 0.3 mM H_2O_2 with or without 10μM PJ-34 for 30 min. Then ATF4 was immunoprecipitated and subsequently ADP-ribosylation of the protein was detected by immunoblot analysis using an poly(ADP-ribose) antibody. Analyzing the ADP-ribosylated ATF4 verified that oxidative stress induced PARP-1 activation trigger ADP-ribosylation of ATF4 (Fig.4).

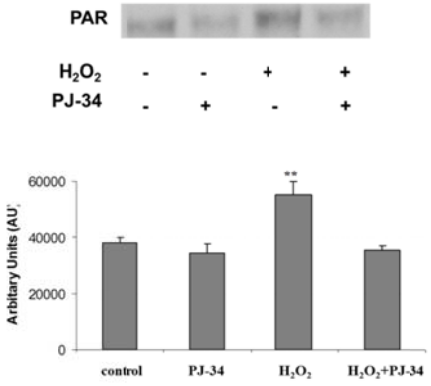

Fig.4.

DNA binding capacity of ATF4 under oxidative stress induced ADP-ribosylation

DAPA experiments were performed to analyze the ATF4 transcription factor binding to the respective CRE in 0.3 mM H_2O_2 treated cells with or without 10μM PJ-34 for 30 min. Probes containing consensus sequences of CRE and CRE mutation were used to clarify the component binding to the CRE sites. Increases in the binding of ATF4 to the CRE site after PJ-34 treatment and PJ-34, H2O2 co-treatment were identified, but it was decreased under oxidative stress condition (Fig.5.).

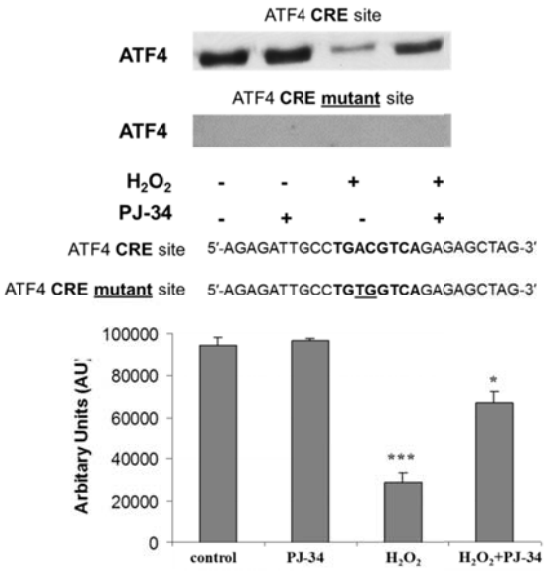

Fig. 5.

Possible model for the cytoplasmic regulation of cell survival by PARP inhibition under oxidative stress via ATF4 and MKP-1.

Cytoplasmic and nuclear events under PARP inhibition (Fig.6.). Arrows: active processes; flat-ended arrows: inhibition; P: phosphorylated.

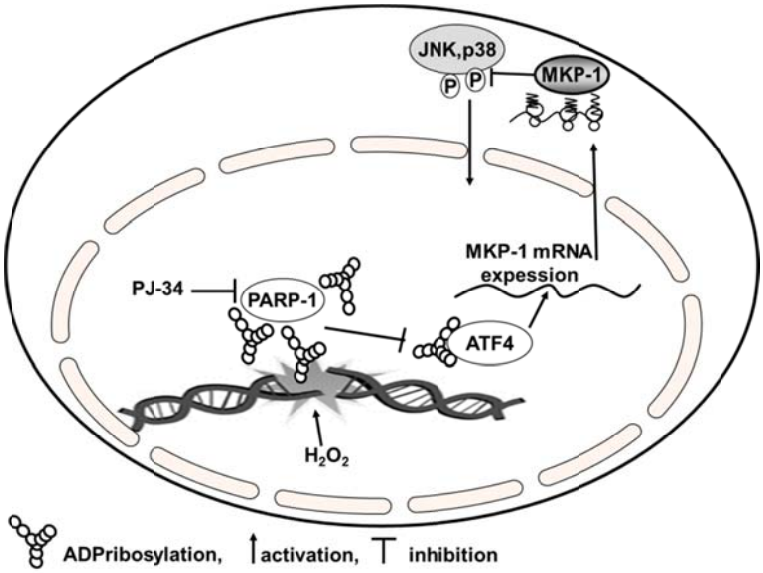

Fig.6.

This grant is given for 3 years, however, due to personal reasons I am unable to continue working on the project from the end of the first year. Therefore, the grant has been ended in accordance with the rules.

Majority of the experiments detailed in the research proposal have been carried out during the first year. Due to premature ending, the yearly scheduling of research proposal has been modified and funds allocated for conference attendance were instead used for purchasing chemicals.

Results of the project will be submitted for publication at a later time; details of accepted publications will be sent to the OTKA committee thereafter.