Inhibition of CDK4 activity by 7-chloro-4-nitro-benzo [1,2,5]oxadiazole 1-oxide

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By reason of cell cycle regulatory function of cyclin dependent kinase (CDK), modulation of CDK activity is very important for anti-cancer therapeutic strategy. For mass screening of CDK4 inhibitor, we set up CDK4 assay in vitro using a cyclin D1-CDK4 fusion protein, which maintains the high enzyme activity and stability. From the screening of representative compound library of Korea Chemical Bank, we found that 7-chloro-4-nitro-benzo [1,2,5]oxadiazole 1-oxide (FBP-1248) selectively inhibited CDK4 activity by ATP competitive manner. This compound also induced Rb hypophosphorylation and inhibited cell growth by the regulation of cell cycle. In summary, we developed efficient CDK4 assay system in vitro and identified a CDK4 inhibitory compound, FBP-1248 by the application of the assay system.

Ischemic induction of caspase11 is attenuated by hypoxic-preconditioning

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The FACs analyses showed that injurious ischemia causes the death of RAW 264.7 cells, but the cells previously experienced to short hypoxic preconditioning were not dead after exposing to injurious ischemia. RT-PCR and western analysis showed that ischemic injury increases level of caspase11. Hypoxic preconditioning attenuated the ischemia-induced expression of both the endogenous caspase11 and the reporter gene driven by caspase11 promoter. Our findings suggest that preconditioning blocks the ischemic induction of caspase11, thereby protecting cells from ischemic injury. In order to find the signaling pathways involved in the repression of caspase11 by hypoxic preconditioning, we cotransfected the reporter plasmid with several genes encoding kinases, including PI3K, Src and p38 MAPK. Our results showed that p38 MAP Kinase but neither PI3K nor Src is involved in ischemic induction of caspase11 reporter genes. However, anything could not decrease caspase11 reporter genes in preconditioning. Interestingly, In hypoxia-inducible factor-1α (HIF-1α) and HIF-1α(Amt) deficient cells, level of caspase11 protein is higher than that in wild type cells. [This study was supported by the Neurobiology Research program grant (2004-01969) from the Ministry of Science and Technology]

Isolation of hypoxia regulatory genes using cell-based functional screening

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Hypoxia-induced transcription factor-1α (HIF-1α) is an oxygen-sensitive protein that becomes stabilized in response to hypoxia, whereas it is degraded through ubiquitin-proteasome system in normoxic condition. Hypoxic condition is induced in some diseases such as cancer, heart disorder and stroke. Thus, excavation of new genes regulating hypoxia is as very valuable research as ever for finding therapeutic targets. In this effort, we screened cDNAs whose over-expression affects the cell survival rate compared with control in oxygen-deficient condition. In addition, we established a simple visual method employing induction of green fluorescence protein (GFP) responding to the stabilization of HIF1α. Several genes were identified to regulate hypoxia-induced cell death and HIF1α-mediated reporter activity under hypoxic condition. Ectopic expression of TSC, which is believed to a tumor suppressor candidate, enhanced hypoxic cell death and suppressed HIF1α-mediated reporter activity. Down-regulation of death domain containing DDPCP protein in HT22 neuronal cells suppressed hypoxic cell death, while its over-expression suppressed the transcriptional activity of HIF1α. Further the mechanisms of these genes in regulating hypoxia will be discussed.

Mitochondrial NADP+-dependent isocitrate dehydrogenase protects cadmium-induced apoptosis

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Cadmium is known to exhibit a high affinity for thiol groups and may therefore severely disturb many cellular functions. Recently, we demonstrated that the control of mitochondrial redox balance and oxidative damage is one of the primary functions of mitochondrial NADP+-dependent isocitrate dehydrogenase (IDPm). When exposed to cadmium, IDPm was susceptible to the loss of enzyme activity and the structural alterations. Site-directed mutagenesis confirms that binding of cadmium occurs to a Cys379 of IDPm. Also we observed a clear inverse relationship between the amount of IDPm expressed in target cells and their susceptibility to cadmium-induced modulation of cellular redox status and apoptosis. When oxalomalate, a competitive inhibitor of IDPm, was administered to mice, inhibition of IDPm and Grx and enhanced susceptibility to apoptosis was observed upon their exposure to cadmium. These results suggest that IDPm plays an important protective role in cadmium-induced apoptosis by maintaining the cellular redox status and by the protection of Grx activity.